AN INVESTIGATION OF A REPEAT BREEDER PROBLEM IN DAIRY COWS

BY

OSMAN VALLI PATEL

A Thesis submitted for a Masters Degree in Veterinary Medicine,

Faculty of Veterinary Medicine,

University of Glasgow.

Departments of Veterinary

Physiology and

Veterinary Anatomy.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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DECLARATION

I, Osman Valli Patel, do hereby declare that the work presented in this thesis is original, was carried out by me and has not been presented for an award of a degree in any other University.

SUMMARY

Repeat breeding in dairy cows is still a major problem and causes substantial economic loss through increase in calving interval and reduction in milk yield. Although factors related to the bull and the environment have been implicated, the majority of the research has shown that the cow plays a major part in the repeat breeding syndrome. Since Lindhal (1966) first reported the use of ultrasonography in detection of pregnancy in sheep, the technique has gained popularity in veterinary medicine as a diagnostic aid. However, due to the additional equipment cost compared to rectal palpation, it has rarely been used on a commercial basis for diagnosis of reproductive disorders in cows. The purpose of this study was to compare the reproductive events in normal and repeat breeder cows using ultrasonography and plasma progesterone concentration.

Cows were divided into two groups and were bled and scanned regularly. Group one consisted of normal cows and group two consisted of repeat breeder cows. The cows that conceived in both groups were observed from day of insemination up to day 42 post-insemination whereas those that did not conceive were only observed till they returned to service again.

All the cows in group one and two cows from group two conceived, though one of the repeat breeder cows had lost an earlier conceptus but held to the next service. One cow from group two lost the conceptus and developed a luteal cyst during the period of observation. Three other cows from group two did not conceive. Two of them returned to service between day 20 and day 23 while the third cow was not seen on heat by the herdsman during the period of observation. In the pregnant cows, the area, height and width of the corpus luteum, as measured by ultrasound, fluctuated throughout the period of observation but the plasma progesterone concentration remained elevated. In the cows that did not conceive, the corpus luteum, as seen by ultrasound, reduced in size, decreased in echogenicity and its outline became blurred as the next oestrus drew nearer. In addition, in these cows the plasma progesterone concentration declined rapidly and reached basal levels as

the next oestrus approached. More follicular activity was evident in non-pregnant cows than in pregnant cows. In the pregnant cows the ovary contra-lateral to the corpus luteum bearing ovary showed more follicular activity. The uterus in oestrus, dioestrus and pregnancy was found to exhibit characteristic images. These included a distinct folding of the endometrium and accumulation of intra-uterine fluid during oestrus and lack of folding and fluid during dioestrus. The embryonic vesicle was seen as early as day 13 in the pregnant cows and the embryo proper was detected by day 20.

This study demonstrated that ultrasonography in collaboration with plasma progesterone concentration was useful in monitoring the reproductive events in both normal and repeat breeder cows. Although additional cost of the equipment currently precludes the complete replacement of bovine rectal palpation by ultrasound, ultrasonography can augment rectal palpation and endocrinology in individual animals that present diagnostic problems. Further studies are needed to confirm, clarify and extend the findings reported and to justify the commercial use of ultrasonography in monitoring herd fertility.

CHAPTER ONE

LITERATURE REVIEW

GENERAL INTRODUCTION.

Reproduction plays an extremely important role in the beef and dairy industries. A cow must conceive and deliver a viable calf regularly during her reproductive lifetime to fulfill her milking and reproductive potential. Reproductive efficiency can be described as a measure of the ability of an animal to become pregnant and produce a viable offspring within a specified period. The efficiency of reproduction of any species depends on the duration of sexual season, the frequency of recurrence of sexual cycles, ovulation rate, the duration of pregnancy, litter size, the suckling period, the age at puberty and the duration of the reproductively active period in the animals life (Hafez, 1966). At best, a cow is only likely to produce a single calf per year so bovine reproduction is less efficient than in other animals eg. dogs, pigs. In addition, a cow will only begin to lactate after calving and milk production will eventually cease unless she calves again (Mackay, 1981). Furthermore, present day economics dictate that animal production be as efficient as possible. This is especially true in Europe, where the European Economic Commission introduced milk 'quotas' in an effort to curb excessive milk production. Thus, the reproductive process is of vital importance to production efficiency and lactation. However, reproductive wastage is a major problem in all livestock production with embryonic mortality accounting for a major portion of this loss.

From a biological point of view the calving rate i.e. calves born per 100 services is perhaps the most appropriate measure of fertility. However, fertility is usually assessed in economic terms by the calving interval, i.e. the period of time between successive calvings. Esslemont (1982) outlined the general targets for breeding management of dairy cattle in the European Community as follows;

365 days calving interval.

300 days in milk.

56 average dry days (none less than 45 days).

80% heat detection throughout the season.

65 days average interval to first service (none less then 45 days)

57% first service conception rate.

85 days calving to conception.

1.6 services per conception.

95% of the herd should be served.

95% of those served should eventually conceive.

55% average conception rate all services.

5% cows failing to conceive.

15% overall culling rate (including one third for failing to conceive).

High fertility and regular reproduction underlie profitable production of milk and beef. Poor reproductive performance exerts its adverse economic effect in many, often interacting ways, as described by Mackay (1981) and Bartlett *et al* .(1986), and includes: prolongation of calving interval; reduced calf sales; postponement of calving time to unfavourable season of the

year; increased age at first calving and reduction in numbers of calves born; additional cost of veterinary services; additional cost of repeated artificial inseminations and reduction of genetic progress due to reduced selection potential.

The financial losses suffered due to infertility are staggering. In the United States, Mackay (1981) estimated the losses to be $300 million and four years later Britt (1985) estimated them to be $1.3 billion. A similar problem exists in Europe. For example in Germany, Zeddies (1982) reported losses upto DM1270 million while Peters and Ball (1987) reported losses up to £290 million in the United Kingdom.

In summary, infertility in cattle is still a major problem in livestock production and causes substantial economic losses in most areas of the world. Before discussing the literature which describes the likely causes of infertility in cows, the normal control of the bovine oestrous cycle and postpartum period will be described.

THE BOVINE OESTROUS CYCLE.

In the non pregnant cow oestrus occurs at approximately 21 day intervals. The cow, like the sow, is a non-seasonal polyoestrous animal and once oestrous cycles are established they continue indefinitely unless interrupted by pregnancy or pathological anomalies. The oestrous cycle is divided into four phases according to the changes that occur during the cycle (Salisbury *et al.*,1978; Hafez,1980, & Arthur *et al.*,1989):-

(i) Pro-oestrus; This phase immediately precedes oestrus and is characterised by a marked increase in activity of the reproductive system. Regression of the corpus luteum of the previous cycle is initiated, accompanied by follicular development. The endometrium becomes congested and oedematous and its glands show evidence of increased secretory activity. The vaginal and cervical mucosae become hyperaemic.

(ii) Oestrus; This is the main reference point of the cycle and is when a cow will stand to be mated. The interoestrus interval is used to define cycle length. During oestrus the uterine, cervical and vaginal glands secrete increased amounts of clear and adhesive mucus which tends to hang from the vulva and frequently adheres to the tail. The cervix is relaxed and patent allowing one or two fingers to be inserted into the external cervical os. The uterus on rectal palpation gives a highly characteristic tone commonly described as "hard rubber" feeling and the horns are tightly coiled. During pro-oestrus and oestrus the follicle which is soon to rupture enlarges and on rectal palpation of the ovaries it is usually possible to detect the ripening follicle as a bulging, smooth soft area on the surface of the ovary. The corpus luteum of the previous cycle undergoes rapid reduction in size and the protrusion of it from the ovarian surface becomes less distinct. The animal by now will be restless and more active. It spends less time eating, resting and ruminating and this subsequently reduces milk yield. She tends to lick and sniff the perineal region of other cows and will attempt to mount them. In addition she will tend to butt other cows and when mounted by them she will stand frequently raising her tail and arching her back. The change in steroidogenic activity of the follicular cells is responsible for these behavioural manifestations, oestrogens being the most important. Pro-oestrus and oestrus are frequently referred to collectively as the follicular phase of the cycle. Ovulation typically occurs about 12 hours after the end of oestrus.

(iii) Metoestrus; This phase succeeds oestrus and corresponds to the period of formation of the corpus luteum, which occurs mainly from differentiation and reorganisation of the follicular granulosa cells. There is a reduction in the amount of secretion from the uterine, cervical and vaginal glands. The external cervical os constricts and the mucous becomes scanty, sticky and pale. Occasionally,

the mucous discharge is blood tinged. Later, the mucous instead of flowing out of the vulva forms a cervical plug that prevents bacteria or other harmful agents from entering the uterus.

(iv) Dioestrus; (Literally means between oestrus). This is the longest phase of the cycle and it is characterised by presence of a functional corpus luteum which secretes mainly progesterone. As the corpus luteum enlarges it tends to push itself out of the ovary and can be detected *per rectum* as a distinct projection on the surface of the ovary. In the majority of cases it is felt like a distinct bulge which tends to be constricted towards its attachment on the ovary. The corpus luteum maintains its maximum size and remains unaltered in appearance until the onset of pro-oestrus. The uterus feels flaccid on rectal palpation. The uterine glands undergo hyperplasia and hypertrophy, the cervix becomes constricted and tightly closed, the secretions of the genital tract are scant and sticky and the vaginal mucosa becomes pale. Metoestrus and dioestrus are frequently referred to as the luteal phase of the cycle.

ENDOCRINE REGULATION OF THE OESTROUS CYCLE.

Extensive review of the complex regulation of cyclical ovarian activity has taken place in the last decade (Ireland and Roche,1982; Schallenberger *et al.*,1984; Walters & Schallenberger,1984; Peters,1985; Ireland and Roche,1987 & Johnson and Everitt,1988) and mainly involves the hypothalamus, the pituitary gland and the ovaries (the so called hypothalamo-pituitary-ovarian axis). The hypothalamus controls gonadotrophin release from the anterior pituitary by secreting a releasing factor known as gonadotrophin releasing hormone (GnRH). GnRH is carried to the anterior pituitary by the portal vessels and is responsible for stimulating pituitary synthesis and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). GnRH is secreted in a pulsatile or episodic pattern

from the hypothalamus, most pulses being followed by pulses in FSH and LH secretion (Clark and Cummin,1985). In addition, there are two modes of secretion. A tonic mode is responsible for the continuous basal secretion of gonadotrophins and a surge mode controls the short lived massive secretion which causes ovulation. FSH may be regarded as the initiator of follicular growth but it also stimulates the establishment of LH receptors in follicular cells. These LH binding sites are critical for successful entry of the antral follicle into the pre-ovulatory phase of follicular growth. FSH itself principally binds to the granulosa cells of the developing follicle. Under the influence of FSH the maturing follicle increases in size and production of steroids increases. The theca cells synthesize the androgens which are transferred to the granulosa cells and are aromatised to oestrogens (Lacroix *et al.*,1974). A peak in plasma FSH concentration occurs which coincides approximately with oestrus and is followed by a second less well defined peak about 24 hours later (Dobson,1978). During the remainder of the cycle, plasma FSH concentrations appear to fluctuate over periods of about 5 days and the peaks have been associated with waves of follicular development occurring throughout the luteal phase of the cycle (Dobson *et al.*,1973).

The primary function of LH appears to be to stimulate follicular maturation and ovulation followed by formation and maintenance of the corpus luteum. Unlike FSH, LH binds to the theca cells and initiates synthesis of androgens. Mean plasma LH concentrations are low for most of the oestrous cycle with a peak or surge of secretion occurring at about the time of oestrus and coinciding with the first FSH peak (Akbar *et al.*,1974). The LH surge on a pre-ovulatory follicle has two effects. Firstly, it synergises with FSH in causing maturation of the follicle and initiates ovulation. Secondly, it changes the whole endocrinology of the follicle which becomes a corpus luteum. The precise

cell composition of the corpus luteum is uncertain but includes both the theca and granulosa cells. In many species, a carotenoid pigment, lutein, gives the corpora lutea a yellowish or orange tinge. The transformation from follicle to corpus luteum is referred to as "luteinization". Furthermore under the influence of LH the theca and granulosa cells no longer synthesize oestrogens but now produce progesterone. LH is essential for maintenance of the biochemical and morphological integrity of the corpus luteum and is released in high amplitude low frequency pulses resulting in a low average plasma concentration. During the follicular phase this changes to a low amplitude high frequency pattern resulting in higher plasma LH concentration (Rahe *et al.*,1980).

The principal biologically active oestrogen is oestradiol 17 Beta. Other oestrogens eg. oestriol and oestrone can be detected in the body fluids but they are less active metabolites of oestradiol. Oestrogens are synthesized in the ovarian follicle by the two cell mechanism mentioned earlier. The concentration of oestradiol is low in peripheral plasma for most of the oestrous cycle then rises during pro-oestrus and reaches a peak on the day of or the day before standing oestrus. This peak usually just precedes or may coincide with the pre-ovulatory LH peak (Goding *et al.*,1970). Rising plasma oestradiol concentration is correlated with increasing size of the dominant follicle, which is the source of most of the oestrogen (Ireland and Roche,1987). Oestrogen is responsible for the signs of behavioural oestrus. The high oestradiol concentrations during the follicular phase of the cycle induce the pre-ovulatory gonadotrophin surge by a positive feedback mechanism acting on the hypothalamo-pituitary axis (Goodman and Karsch,1980). This mechanism increases the frequency of hypothalamic GnRH secretion and the responsiveness of the anterior pituitary (Goodman and Karsch,1980).

Progesterone is the major steroid secreted by the corpus luteum and plasma concentrations reflect luteal development, maintenance and regression. Plasma progesterone concentrations begin to rise from day 4 of the cycle, reaching a peak around day 8 and remaining high until day 17. Concentrations then decrease to basal levels before the next oestrus and ovulation (Peterson *et al.*,1975). High progesterone concentrations during the luteal phase exert a negative feedback inhibition on gonadotrophin release. It also inhibits uterine activity and stimulates uterine glandular development. As the corpus luteum begins to regress 3-4 days before oestrus, plasma progesterone concentrations decrease and pituitary gonadotrophin secretion rises as negative feedback is removed. This stimulates follicular maturation and increased oestradiol secretion. Increasing oestradiol concentrations eventually trigger the LH surge (Goding *et al.*,1970). Removal of the negative feedback effect of progesterone secreted from the corpus luteum is the key event in initiation of another oestrous cycle.

It is generally recognised that in the cow, as in other species, a substance of endometrial origin causes luteal regression . In cattle and sheep this effect is local and the uterine horn ipsilateral to the corpus luteum is required to cause luteolysis (McCracken *et al.*,1973). Prostaglandin F2a (PGF2a) has been shown to have potent luteolytic activity in the ewe, doe, cow, sow and mare (Auletta and Flint, 1988). In the sheep and cow, PGF2a is released from the endometrium into the uterine vein, which passes very near to the ovarian artery. PGF2a is transferred directly from vein to artery by diffusion and hence is conveyed to the corpus luteum. The mechanism by which PGF2a causes luteolysis is not fully understood but there are several possibilities, PGF2a interacts directly with the luteal cells and indirectly via a reduction in luteal blood flow (McCracken *et al.*,1984; Auletta and Flint,1988 & Knickerbocker *et al.*,1988). PGF2a directly affects the structure and function of luteal cells by allowing high levels

of free calcium ions to accumulate intracellularly by reducing the activity of the ionic pumps. Sustained elevations of intracellular free calcium concentrations are cytotoxic in numerous cell systems, suggesting that the luteolytic effect of PGF2a is mediated, in part, by the intracellular concentrations of calcium (Auletta and Flint,1988 & Knickerbocker *et al.*,1988). In addition, it has been found to block the LH receptors on the steroidogenic cells of the rat corpus luteum and this in turn reduces the amount of progesterone produced. It is envisaged to act in a similar way in ruminants but this has yet to be clarified. In terms of indirect effects, PGF2a is vasoactive and exogenous prostaglandin dramatically decreases blood flow to the corpus luteum bearing ovary. A similar decrease in luteal blood flow occurs during normal luteolysis. However, it is not yet clear whether the decrease in luteal blood flow is actually the cause of luteolysis or whether it is a consequence of other processes involved in luteal regression. PGF2a is released in a pulsatile manner starting about day 17 of the cycle and continuing for a 2-3 day period or at least until plasma progesterone concentration is minimal. The hormonal mechanisms initiating PGF2a release are not fully understood, but it is thought that a period of oestrogen and progesterone priming is required before PGF2a is secreted. In addition, there is now a good deal of evidence indicating that oxytocin is synthesized and secreted by the corpus luteum in ruminants, and that its secretion by the ovary is involved in the control of the pulsatile release of uterine PGF2a at the end of the non-pregnant oestrous cycle (Flint and Sheldrick,1982). This evidence includes the prolongation of the oestrous cycle in sheep following immunisation against oxytocin, the shortening of the oestrous cycle after administration of oxytocin in some animals, oxytocin release in response to PGF2a injection and the synchronous secretion of oxytocin and PGF2a at luteolysis (Flint and Sheldrick,1983).

Prolactin, which is also secreted from the anterior pituitary, is an essential part of the luteotrophic complex in the rat. It was thought to play a similar role in the ewe. However, recent experiments using the dopamine agonist Bromocryptine, which is a potent inhibitor of prolactin secretion in cattle and sheep, did not appear to influence the length of the oestrous cycle or synthesis of progesterone (Auletta and Flint,1988). From this Auletta and Flint (1988) concluded that prolactin is neither necessary for normal luteal function nor involved in luteolysis in the ruminants.

Inhibin, a protein hormone, produced by the granulosa cells, has been found to have an important role in regulating pituitary FSH secretion in both males and females (Henderson and Franchimont,1983 & Demoulin *et al.*,1987). In the cow, plasma inhibin concentration is directly correlated to the follicular diameter. This probably explains how growth of additional follicles is inhibited by a dominant follicle. The regulation of inhibin production is still unclear but androgens incubated with granulosa cells *in vitro* were found to stimulate inhibin production whereas progesterone was found to inhibit inhibin production (Henderson and Franchimont,1983). Oestrogens were found to have no significant effect.

ESTABLISHMENT OF PREGNANCY.

Establishment of pregnancy involves interactions between the conceptus and the dam. The critical nature of the peri-attachment period and the necessity for synchrony between embryo and uterus emphasizes the importance of both uterine environment and conceptus "signals" in recognition of pregnancy (Thatcher *et al.*,1984). One important role of the conceptus is to provide signals which allow persistence of the corpus luteum for an extended period in order to maintain circulating concentration of progesterone sufficient for maintenance of

pregnancy. The process by which the peri-attachment conceptus signals its presence to the maternal unit, as reflected by luteal maintenance, has been referred to as "maternal recognition of pregnancy" and the bovine conceptus signals its presence by day 16-18 (Northey and French,1980 & Thatcher *et al.*,1984). The bovine conceptus produces some testosterone, progesterone, oestradiol, prostaglandins, proteins and possibly other unidentified agents (Shemesh *et al.*,1979; Northey and French,1980; Janzen *et al.*,1982 & Thatcher *et al.*,1984, 1989). Much recent work has centred on the proteins secreted by the pre-implantation embryo. Bovine trophoblast protein-1 (bTP-1) is the major protein secreted by the bovine embryo and has similar properties to those of ovine trophoblast protein-1 (oTP-1) (Bartol *et al.*,1985). Bovine trophoblast protein-1 is secreted at a time corresponding to the time of maternal recognition of pregnancy and is thought to be the principle signal for maternal recognition of pregnancy (Roberts *et al.*,1990). Based on recent experiments *in vitro*, Thatcher *et al.*(1989) found that bTP-1 exerts anti-luteolytic effect on the uterus by decreasing the secretion of PGF2a and this results in maintenance of the corpus luteum. Gross *et al.*(1988) suggested that bTP-1 induced peroxidase activity in the endometrium which inhibited intracellular prostaglandin synthesis. Flint *et al.*(1989) reported that oTP-1 reduced the concentration of oxytocin synthesized and secreted from the corpus luteum and in addition, reduced the uterine concentration of oxytocin receptors, suggesting that oTP-1 reduced production of PGF2a by blocking other mechanisms which encouraged its production. In addition, incubation of conceptus tissue *in vitro* indicated that the blastocyst directed biosynthesis from PGF2a to PGE2 (Lewis and Waterman,1983). Henderson and McNatty (1975) suggested that PGE2 counteracted the luteolytic effect of PGF2a. However, McCracken *et al.*(1984) found that PGE2 on its own was not completely able to protect the corpus luteum against a luteolytic dose

of PGF2a. But it may be possible that PGE2 either just contributes to or reinforces the predominately luteotrophic role of bTP-1 (Thatcher *et al.*,1989). Oestrogens derived from the trophoblast act as a major signal associated with pregnancy recognition and luteal maintenance in the pig (Bazer and First,1983). However, Ottobre *et al.*(1984) reported that increases in oestrogens were not involved in regulating or initiating maternal recognition of pregnancy in sheep. Their precise role in cattle has not yet been determined.

The average length of gestation in cattle is 280 days and rectal palpation is the most commonly used method for pregnancy diagnosis (Wisnicky and Casida,1948). The amniotic vesicle can be felt slipping away between the thumb and forefinger as early as day 30 and by day 40 the pregnant horn is markedly enlarged compared to the non-pregnant horn (Wisnicky and Casida,1948). The fetus is palpable between day 60-70 (Hancock,1962) and by day 90 the cotyledons can be palpated (Fincher,1943). In addition, the uterine artery on the pregnant side displays a characteristic fremitus from day 90 onwards. The pregnant uterus starts to descend towards the abdominal floor from the fourth month onwards and it is difficult to palpate the fetus from then on. The fetus is palpable once again from the eighth month onwards and at this time the different regions of the fetus are palpable.

In summary, detailed knowledge of the hypothalamo-pituitary-ovarian axis is important in order to understand the normal reproductive process. However, it seems clear that the endocrine control of the oestrous cycle in domestic animals is more complicated than originally hypothesized. As more research work unfolds on the basic mechanisms of endocrine control of the normal oestrous cycle, it may provide valuable information in explaining the causes of early pregnancy wastage.

THE POST-PARTUM PERIOD

In most mammals, pregnancy and parturition is followed by an indefinite period of ovarian inactivity and sexual quiescence called the postpartum anoestrus. Lactation is an important factor controlling the length of this anoestrus phase although there are important species variations in the extent of anoestrous during lactation. Thus pigs remain anoestrous during lactation whereas in cattle, cycles are reinitiated quite early in lactation. The length and management of the post-partum phase is a critical determinant of efficient reproduction in the cow since a long period of non-pregnancy will have a deleterious effect from an economic point of view. Re-establishment of reproductive activity must compete with the restorative processes in other body systems recovering from pregnancy and parturition as well as with the metabolic requirements of lactation (Karg and Schallenberger, 1982). In order that the fertility of an individual cow and of the herd as a whole is maintained, it is important that pregnancy, parturition and the puerperium are normal. During pregnancy the genital system undergoes certain changes in order to accommodate the developing embryo/fetus and to aid in expulsion of the fetus (Morrow *et al.*,1969 & Noakes, 1984). After parturition, such changes are reversed, a process which is not completed immediately and which is quite variable between animals. Karg and Schallenberger (1982) and Peters (1984) outlined the following factors which affect the duration of postpartum anoestrus;

(1) UTERINE INVOLUTION. Literally means rolling inwards or turning in and describes the return of the uterus from gestational to cyclic dimensions. Opinions differ as to the time required for completion of involution although it is generally accepted that the uterus never returns to its previous non pregnant size. This is due to small incremental increases which occur with each successive pregnancy (Moller, 1970). The same author expressed

difficulty in detecting much change in uterine size after 25 to 30 days *post partum*. There is considerable loss of maternal tissue during parturition and placental separation and therefore complete regeneration of the endometrium is necessary before the next implantation. Although involution is thought to be complete in gross terms by 25 to 30 days *post partum*, restoration of the normal microscopic structure, particularly that of the epithelium, may take as long as 50-60 days (Noakes,1984). A number of factors delay involution and endometrial repair as reviewed by Morrow *et al.*(1969), Vandeplassche and Bouter (1982) & Noakes (1984);

(a) Age. It is generally assumed that involution occurs more rapidly in primipara than pluripara.

(b) Season of the year. Involution occurs more rapidly in cows calving in spring and summer than in autumn and winter.

(c) Climate. Heat stress delays uterine involution.

(d) Periparturient problems. Dystocia, retained placenta, hypocalcaemia, ketosis, twin calves and uterine infections can all prolong uterine involution. Retained placenta or uterine infection probably delays regeneration of the endometrium.

(e) Bacterial contamination. The vulva, vagina and cervix, which comprise the physical barriers to the entry of harmful micro-organisms into the uterus, are breached at parturition (Schirar and Martinet,1982). Noakes (1984) outlined the following factors as the most important in the elimination of the bacteria;

(i) Return to normal cyclical ovarian activity with the increased resistance of the oestrogen dominated uterus at oestrus.

(ii) Normal uterine involution and endometrial repair.

(iii) Removal of damaged or devitalised tissue.

(2) MILK PRODUCTION. Longer acyclic periods have been observed in high yielding dairy cows. Cystic ovarian disease is also more common in high yielding dairy cows.

(3) NUTRITION. Inadequate feeding during the dry period and after calving delays post-partum ovarian activity (Sejrsen and Neiman-Sorensen,1982).

(4) BREED. Beef cows have a longer period of acyclicity than dairy cows. There are considerable variations within and between breeds.

(5) SEASON OF THE YEAR. Studies have shown that ovulation is delayed longer in spring calving cows than those calving at other times of the year. Recent evidence suggests that photoperiodicity affects the return of normal ovarian function.

(6) CLIMATE. High environmental temperatures have a profound detrimental effect on oestrous cycle length as well as intensity of oestrus (Gwazdauskas,1975 & Zoldag,1983).

(7) SUCKLING AND FREQUENCY OF MILKING. The most important influence on the return to normal cyclical activity relates to the mode of milk removal (Noakes, 1984 & Peters, 1984). Though suckling enhances uterine involution in cows, milked cows return to oestrus earlier than suckled cows.

Optimal reproductive activity in post-partum cows is achieved when they experience phases of endocrine activity which culminate in ovulation of a viable egg. Additionally, ovulation should be preceded by well defined and synchronous manifestations of oestrus and followed by the development of adequate luteal function (Lamming *et al.*,1982).

The bovine ovaries are not quiescent during pregnancy. The average diameter of the largest follicles decreases from 12mm in the second month of pregnancy to approximately 9mm in the fifth month and to 4mm in the eighth or ninth month (Schirar and Martinet,1982). By the time of parturition, follicular diameters are minimal. However, Kessler *et al.*(1980) & Webb *et al.*(1980) used rectal palpation and were able to detect ovarian follicles 5 to 10mm in diameter as early as 4 to 5 days post-partum. By day 10 *post partum* the ovaries increase in size and weight (Callahan *et al.*,1971). Savio *et al.*(1990) using ultrasonography reported presence of a dominant follicle as early as day 11. Most authors agree that by around day 15 *post partum*, follicles ready to ovulate are present (Wagner and Oxenreider,1971; Stevenson and Britt,1979 & Kessler *et al.*,1980). However, such follicles appear to undergo atresia because ovulation at this time interval *post partum* occurs only sporadically. Subsequently, other follicles develop to mature size and again become atretic. First ovulation occurs approximately 25 to 30 days *post partum* (Schirar and Martinet,1982) but is not accompanied by oestrus because progesterone is required to prime the central nervous system and behavioural centres (Hafez,1980). First standing oestrus in the dairy cow usually occurs about 40-50 days *post partum* (Schirar and Martinet,1982).

Lamming *et al.*(1982) & Peters and Lamming (1984,1986) have postulated the following sequence of endocrine events in the post-partum cow;

(i) Some GnRH is secreted immediately *post partum* but its quantity and/or the frequency with which it is secreted is inadequate to release sufficient quantities of gonadotrophins to induce cyclic ovarian activity.

(ii) Plasma FSH concentrations rise rapidly after parturition, stimulating follicular development.

(iii) There is a gradual increase in the frequency of LH pulses and in plasma LH concentration as the effects of the high plasma progesterone concentration of pregnancy are withdrawn.

(iv) Gonadotrophin secretion stimulates follicular growth and the production of oestradiol and perhaps inhibin.

(v) Concurrent with these changes there is a gradual recovery of the oestradiol positive feedback mechanism.

Other endocrine organs have also been implicated in the control of gonadotrophin secretion *post partum.* Kindahl *et al.*(1982) found that PGF2a is released from the uterus for a considerable time after parturition and this seems to be related to the time required for completion of uterine involution. Plasma corticosteroid concentration is increased by such stimuli as suckling, stress or disease and is known to inhibit secretion of LH (Wagner and Li,1982). Maule-Walker *et al.*(1983) reported that the mammary gland also secretes PGF2a and Oestradiol 17 Beta in the periparturient period.

To summarize, a number of factors acting either individually or cohesively affect the length of the post-partum period. The length and management of this period are critical determinants of efficient and economical animal production and as reviewed by Esslemont (1982) and discussed earlier, onset of normal oestrous cycles and high first service conception rates are critical to achieving the 365 day target for calving interval. One of many potential conditions which can compromise breeding efficiency is the repeat breeder syndrome.

THE REPEAT BREEDER COW.

Introduction. Repeat breeder syndrome can be defined as a condition in which cows or heifers exhibiting regular oestrous cycles and appearing normal on clinical examination have failed to conceive following three or more services (Roberts,1971; Zemjanis,1980 & Noakes,1988).

The problem of repeat breeding is recognised world-wide but the incidence rate varies from one area to another eg. 10% in Sweden (Hewett, 1968), 5% in Israel (Francos,1974), 22% in Bangladesh (Rahman *et al.*,1975), 20% in Scandinavia (Roine and Saloniemi,1978), 22% in India (Singh *et al.*,1981). Lafi and Kanene (1988) estimated an overall incidence rate of 10-25%. Although the repeat breeding problem is usually only temporary and many of the affected cows (approximately 60%) usually conceive normally by the fourth insemination (Dekruif,1976 & Bulman and Lamming,1978), the principal losses are economic. A survey of dairy producers in the north eastern United States conducted by Steele *et al.*(1981) revealed that 2.4% of the culled cows were repeat breeders. In a later study, Bartlett *et al.*(1986) reported that 23.9% of the culled cows in the United States were repeat breeders. In addition, the same author estimated a loss of $1000 per cow culled as a repeat breeder. Mackay (1981) reported that 10-15% of the total loss of $800 million was due to repeat breeders. The lactation losses with repeat breeding were approximately $386 per cow (Bartlett *et al.*,1986).

Causative factors. The reproductive cycle consists of a sequence of many well synchronised events which includes follicle maturation, oestrus, ovulation, sperm transport, sperm capacitation, fertilisation, zygote transport, endometrial progestation, blastocyst expansion and hatching from zona pellucida, implantation, placental formation, embryonic differentiation, fetal development, parturition and lactation. The intricate endocrine interaction between the hypothalamo-pituitary-ovarian axis and also the reproductive organs,

control these processes. Lack of integration of any phase of the hormonal sequence will result in reproductive failure. Genetic, anatomical, environmental, nutritional, metabolic, immunological and pathological factors also contribute to such failures (Hafez,1966).

The causes of repeat breeding in a cow are complex and are probably most easily considered to involve interactions between the cow, the bull and the cow's environment or management.

(I) COW- RELATED FACTORS.

(a) Age. Many researchers have shown that the incidence of repeat breeders is higher in older cows than the younger ones (Hewett,1968; Dekruif,1978 & Bartlett *et al.*,1986). Erb *et al.* (1985) reported that increasing age was associated directly with an increased risk of veterinarian assisted dystocia, retained placenta and metritis. Dekruif (1978) observed a difference of 5% in the pregnancy rate between primiparous and pluriparous cows.

(b) Genetic factors. The use of a bull that produces large calves increases the probability of causing dystocia and post-partum metritis which may cause repeat breeding and infertility. Linares *et al.*(1980) observed that abnormal embryos occurred more frequently in repeat breeders than in virgin heifers. Embryonic morphology is very important for survival in the early days of pregnancy (Linares,1982 & Gustaffson and Larsson,1983). Linares (1982) compared the development of embryos from repeat breeder heifers with that of embryos from virgin heifers at seven days after standing heat and found that two of the repeat breeders were heterozygous for 1/29 chromosomal translocation and one had X-trisomy.

In these heifers there was a tendency to a higher incidence of fertilisation failure and morphologically deviant embryos than in normal heifers. Similar results were found by King and Linares (1983).

(c) Anomalies of the reproductive tract. Roberts (1971) classified these anomalies into two categories;

(i) congenital anatomical defects.

(ii) acquired anatomical defects.

Gustaffson *et al.*(1985) found that two repeat breeders with 1/29 chromosomal translocation had congenital abnormalities of the uterus and cervix. Of the acquired anatomical defects, oviducal obstruction is one of the most important causes of repeat breeding. Although cows with unilateral oviducal obstruction may conceive, bilateral obstruction prevents conception (Kelly *et al.*,1981 & Boyd *et al.*,1984). Oviducal and ovarian bursal adhesions occur especially after manual corpus luteum enucleation and caesarean section (Dawson,1958 & Bowen *et al.*,1978).

(d) Fertilisation failure and early embryonic death. Both fertilisation failure and early embryonic losses are major causes of repeat breeding (Casida,1961). Fertilisation failure may result from death of the sperm or ovum, structural and functional abnormalities in either the ovum or the sperm, immunological incompatibility between ovum and sperm, acquired lesions of female reproductive tract, anovulation or delayed ovulation, or a hostile uterine environment (Hafez,1966 & Noakes,1988). Early embryonic death can result from factors such as nutritional deficiencies, endocrine deficiencies or imbalance, infections, genetic incompatibility and stress (Noakes,1988). In repeat breeder cows the fertilisation rate and embryo survival rate may be as low as 56% and 23% respectively (Graden *et al.*,1968 & O'Farrell *et al.*,1983). The time of occurrence of embryonic mortality in

repeat breeder cows is inconsistent. Hawk *et al.* (1955) suggested that the major portion of embryonic loss occurs between days 16 and 34 after insemination. On the other hand, Ayalon (1978) suggests day 7 as the critical day on which the major portion of embryo deaths occur. Further data from Almeida *et al.*(1984) involving embryo transfers to normal and repeat breeder cows at six days after oestrus, records pregnancy rates of 39% and 10% respectively. Ayalon (1978) reported embryo viabilities of 83% at 2-3 days, and 69% at 35-42 days in normal dairy cows, whereas for repeat breeders the equivalent recovery rates were 71% and 35% respectively. Linares *et al.*(1980) using non-surgical collection 7 days after standing heat reported a recovery rate of 72% for normally ovulating virgin heifers and 57% for repeat breeders. Linares (1982) while recording a fertilisation rate of 89% in repeat breeder heifers found that by day 7, only 28% of these embryos were classified as morphologically normal.

(e) Hormonal factors. The early recognition of pregnancy in cattle involves a series of embryonic generated signals to the mother which are believed to prevent the onset of luteolysis and thereby permit the continued development of the corpus luteum of pregnancy. The embryo, inherently and in response to secretory products of the uterus, develops and grows. However, the need for synchrony between the developing embryo and secretions of the uterus has long been recognised as critical to the maintenance of a successful pregnancy and considerable embryonic mortality occurs when uterine secretions become altered in such a manner that they are asynchronous to the developing embryo. Randal *et al.*(1971) noted that repeat breeder cows had low levels of oestrogens but Erb *et al.*(1976) found no difference. Kodagali *et al.*(1979) postulated that the low levels of LH in repeat breeders were unable to induce complete luteinisation and this led to inadequate corpus luteum

function. Casida (1961) and Bulman and Lamming (1978) suggested that repeat breeding could be caused by an inadequate supply of progesterone. In this context, Maurer and Echternkamp (1982) observed a lower percentage of embryonic survival in beef cows with low serum concentration of progesterone during the period of corpus luteum formation i.e. metoestrus. The same authors reported that a preovulatory LH peak of low amplitude and a long interval from the onset of oestrus to the LH peak, was likely to result in a low serum progesterone concentration during metoestrus. This led them to conclude that hormonal asynchrony was the possible cause of abnormal embryos. Gustaffsson (1985) also reported that retarded embryonic development was due to hormonal disorders at oestrus. However, Kindahl *et al.*(1976) and Linares *et al.*(1982) and later work by Echternkamp and Maurer (1983) showed that lower pregnancy rates were not directly associated with low systemic progesterone concentration.

The utero-embryonic interaction is very complex and more work needs to be done to elucidate the mechanism involved in recognition of pregnancy and maintenance of the corpus luteum. The present understanding of utero-embryonic biochemical interactions is incomplete.

(f) Infectious agents. Infections of the bovine genital tract affect fertility by altering its environment. This may impair sperm transport or viability; cause death of the embryo or fetus or result in still births or weak calves (Hafez, 1966). Infections occur naturally and may be specific or non-specific (Archbald,1977).

Specific infections develop without predisposing causes and are of the enzootic type (Merchant and Barner,1978). Examples of important diseases in this group include brucellosis, campylobacteriosis (Vibriosis), leptospirosis, trichomoniasis, rift valley fever, bovine viral

diarrhoeal/mucosal disease, and infectious bovine rhinotracheitis. These diseases are important economically because they induce abortion and may be the cause of subsequent infertility (Roberts,1971). Diseases such as trichomoniasis, campylobacteriosis and infectious bovine rhinotracheitis cause early embryonic death (Gillespie and Timoney,1981).

Non-specific infections require a predisposing cause eg. poor obstetrical practise, and tend to affect individual cows. The organisms involved are mainly opportunistic and examples include *Staphylococcus, Streptococcus, Actinomyces (corynebacterium) pyogenes* and *Escherichia coli* (Namboothiripad and Raja,1976; Awad and El-Hariri,1980 & El-Nagar *et al.*,1983).

Both specific and non-specific organisms can reach the uterus either via the blood stream or through the vagina (Barbu and Rus,1980). These micro-organisms show an affinity for the pregnant uterus and may directly attack the fetus or cause damage to it indirectly by way of inflammatory lesions in the fetal and maternal placenta. These reactions lead to death and abortion of the fetus, and a higher incidence of retained placenta leading to endometritis (Vandeplassche and Bouters,1982). More specifically, leptospirosis causes degeneration and focal necrosis of the villi, oedema of the intercotyledonary tissue and lymphocyte infiltration (Murphy and Jensen,1969). In experimental leptospiral infection (Morter *et al.*,1958) lesions occurred in the maternal placental crypts which led to separation, fetal death and abortion. Ellis *et al.*(1976) found degenerative changes in the kidneys, liver and blood vessels of the aborted fetus. Similarly, with chronic infection, the endometrium may become severely damaged and this may lead to permanent sterility.

(g) Metritis and endometritis. Both of these have an adverse effect on the

reproductive performance of dairy cattle (Sandals *et al.*,1979 & Miller *et al.*,1980). Metritis and endometritis are common sequelae to placental retention (Sandals *et al.*,1979 & Erb *et al.*,1985). Some correlation between incidence of endometritis and repeat breeding has been reported (Seitaridis and Tsangaris,1973; Barbu and Rus,1980 & Borberry and Dobson,1989). Histological examination of material collected by endometrial biopsy from repeat breeder cows revealed abnormal uterine glands, endometrial hyperplasia and infiltration by inflammatory cells and desquamation of endothelium (Cupps,1973 & Sinha *et al.*,1983). Bugalia *et al.*(1988) found that the concentration of glycogen, protein, acid phosphatase, alkaline phosphatase, DNA and RNA were significantly higher in the endometrium of fertile cows than in repeat breeder cows. Severe sclerosis of the endometrium caused by eg. caesarean section, can also lead to permanent sterility (Vandeplassche and Bouters,1982).

(h) Dystocia. Dystocia delays uterine involution and thus decreases reproductive efficiency by increasing the number of days to first service as well as the number of services per conception (Vandeplassche and Bouters,1982 & Noakes,1984).

(i) Milk fever and ketosis. No direct correlation has been found between these and repeat breeding. However, they may affect other reproductive parameters indirectly (Curtis *et al.*,1983 & Erb *et al.*,1985).

(II) BULL-RELATED FACTORS.

The majority of dairy cattle world-wide are inseminated artificially. This controls the spread of venereally transmitted diseases. Shannon (1978) noted that the number of sperms used, sperm survival rate, time of insemination relative to oestrus, location of semen deposition in the female tract, as well as a number of environmental and management factors,

are involved in determining the success of insemination and, therefore the rate of return to service. Other authors list similar variables which govern the success of artificial insemination eg. Moller *et al.*,1972, Watson and MacDonald,1984 & Gwazdauskas *et al.*,1986. So care should be taken to ensure that an apparent repeat breeder problem is not just a problem related to insemination technique.

(III) ENVIRONMENT AND MANAGEMENT RELATED FACTORS.

(a) Nutrition. The inter-relationship between nutrition and reproduction in dairy cows is a topic of increasing importance and concern among veterinarians and dairymen. Several authors have shown direct, indirect or a combination of direct and indirect evidence of nutrient involvement with post-partum diseases (Curtis *et al.*,1985). Calving difficulties and metabolic disorders, especially milk fever, ketosis, retained placenta and metritis occur more frequently in fat cows and lead to increases in the number of inseminations per conception (Gerloff and Morrow, 1986). Cows receiving excess dietary protein required more services per conception (Davison *et al.*,1964 & Jordan and Swanson,1979). More recently Maree (1986) reported that dairy cows fed high levels of concentrates had a higher incidence of dystocia and a longer duration of parturition. Carson *et al.*(1978) reported that higher than normal plasma levels of phosphorus and calcium increased the probability of dystocia, retained placenta and post-partum metritis but Kumar *et al.*(1986) found that serum concentrations of phosphorus and calcium were lower in repeat breeder cows than in normal cows. This suggests that the concentration of phosphorus and calcium in serum has to be within an optimum range for proper breeding. Julian *et al.* (1976) and Eger *et al.*(1985) reported that the incidence of retained placenta was higher in selenium

deficient animals. McDonald *et al.*(1961) found that iodine improved conception rates in repeat breeders. Vitamins A and B, copper, cobalt, zinc, manganese and magnesium have all been reported to play an active role in reproduction (Hidiroglu,1979 & Ingraham *et al.*,1987) but their involvement in the repeat breeder cow have not been elucidated.

(b) Season. The efficiency of reproduction is not uniform throughout the year, and different views are expressed in the literature as regards seasonal effects. Thus Hewett (1968) observed higher incidence of the repeat breeder syndrome in cows calving during the autumn and winter whereas Coleman *et al.*(1985) reported that cows which calved in spring required a greater number of services than those calving in other seasons. On the other hand, Bartlett *et al.*(1986) found no direct association between the season of calving and the incidence of the repeat breeding. Gwazdaukas (1975), Francos and Mayer (1981) and Zoldag (1983) found that high environmental temperatures were capable of affecting reproduction but its direct relevance to repeat breeding has not been documented.

(c) Herd size. Hewett (1968) showed that repeat breeders are more common in large herds than in small herds. He reported that in small herds 8.5% of the animals failed to become pregnant after four or more inseminations, whereas a 13.1% was reported in the larger herds. De Kruif (1978) found that the interval between parturition and conception was shorter as the size of the herd increased, but that the calving rate after first service decreased as the size of herd increased.

(d) Heat detection. Poor or inadequate heat detection is an important factor contributing to the repeat breeder cow but one which often receives too little attention. Williamson *et al.*(1972) reported that if heat detection was carried out by milkers, in addition to their chores, up to 12% of the cows

presented for insemination were not in heat. O'Farrell (1975) recommended that checks conducted 5 times daily were adequate to detect at least 80% of cows in heat. Bailie (1982) concluded that increasing the frequency of heat detection checks decreased the number of inseminations per conception and decreased the number of days open in dairy cattle.

(e) Time of insemination. With good detection of oestrus the optimal time to inseminate, according to Foote (1979) can be found by the am-pm rule. Cows first seen in oestrus in the morning are best inseminated in the afternoon of the same day while cows first seen in oestrus in the evening should be inseminated next morning. Gwazdaukas *et al.*(1981, 1986) found no difference in rates of conception in cows inseminated early in oestrus and cows inseminated 12 hours after they were first observed in oestrus.

Other factors such as housing system (Gwazdaukas *et al.*,1983), mastitis (Erb *et al.*,1985), lameness (Lucey *et al.*,1986) and interval between parturition and first insemination (Hillers *et al.*,1984) may affect fertility but their direct relevance to repeat breeding has not been documented.

To summarise, repeat breeding in cows is still a major problem in the dairy industry and causes huge financial losses annually. A number of factors related to the cow, the bull and the environment have been shown to influence the repeat breeding syndrome. However, many of the risk factors associated with repeat breeding such as fertilisation failure, early embryonic death, infectious agents, abortions, periparturient diseases, hormonal disorders, metabolic diseases and nutritional imbalances are all associated with the cow and this strongly suggest that the cow plays a critical role in the problem. Even though the bull, and the cow's environment and management have been implicated in contributing to the problem, their importance and relative contribution to the

repeat breeder syndrome still remains obscure. There is still a need for more comprehensive research to alleviate the problem of repeat breeding in cows, and thus reducing the economic wastage and improving the calf crop.

ULTRASONOGRAPHY.

Ultrasound is defined as sound waves of frequencies greater than those audible to the human ear, that is, greater than 20 KHz. Diagnostic ultrasound employs sound waves of much higher frequency usually between 1 and 10 MHz (Barr,1988). The use of ultrasound began in the human field in 1947, as a diagnostic aid in obstetrics (Kings,1974). Since then sonography has become an important tool in human diagnostic medicine and in the last 10-15 years nearly all parts of the human body have undergone sonographic examinations eg. the brain, the eye, the heart, and the major blood vessels. The relative ease with which an ultrasonographic examination can be performed is a primary factor in its popularity. No special contrast material is needed and there is no delay in the presentation of the results (Roberts,1980). The other appealing factor for ultrasonography is its safety. The potential biological effects of high intensity sound waves have been extensively investigated over the past 10 years. There is no doubt that cells can be permanently damaged by continuous exposure to these high intensity sound waves but the pulse echo principle allows for only brief burst of sound (one microsecond duration) followed by a much longer period of echo retrieval (one millisecond duration). No shielding, safety gowns or gloves are necessary (Herring and Bjornton,1989). Ultrasonographic examination presents a large amount of information for immediate diagnostic use eg. the size, depth and width of pathological masses or cysts. In addition, with real time function the viability of a fetus can be judged from the fetal heartbeat and movements (Boyd *et al.*,1988).

Historically, the first use of ultrasound in veterinary medicine was the use of A mode system to detect pregnancy in the ewe (Lindhal,1966). Since then its use as an aid in pregnancy diagnosis has spread to other domestic animals eg. bitch (Helper,1970), sow (Fraser,1968), doe (Lindhal,1969), cow (Mitchell,1973), camel (Schels and Mostafawi,1978), and mare (Fraser *et al.*,1973). In all these studies the transducer was applied transabdominally to detect fetal movement and blood flow in the heart and large blood vessels of the fetus (Lindhal,1969).

With the advent of gray scale, real time, in the late 1970's, sonography gained popularity in veterinary medicine as a diagnostic aid. Its use extended from domestic animals to laboratory animals as well as zoo animals eg. rabbits (Cubberly *et al.*,1982), guinea pigs (Inaba and Mori,1986), pigeons (Peterson *et al.*,1983), gorilla's (Yeager *et al.*,1981,), brown bears (Tachibana,1986), and dolphins (Williamson *et al.*,1990). In small animal medicine ultrasonography has proved itself as a useful adjunct to radiography in the diagnosis of diseases of many internal organs (Bonagura and Pipers,1983 ; Miller and Cartee,1985 & Nyland and Hagar,1985). Similarly, in the large animals, apart from pregnancy diagnosis, the use of real time ultrasonography has expanded to other areas (Cartee and Rumph,1984; Rogers *et al.*,1986 & Ware *et al.*,1986).

Palmer and Draincourt (1980) were the first to outline the use of hand held intra-rectal transducer. This marked a new era in bovine and equine reproductive management. It allowed more direct contact with the reproductive tract and a higher degree of accuracy. The earlier work mainly focused on the mare due to the economics of the horse industry. Pregnancy diagnosis in the mare by rectal palpation is not possible until approximately one month and it is impossible to judge the viability of the fetus. On the other hand using real time ultrasonography, it

is possible to diagnose pregnancy as early as day 14-17 (Chevalier and Palmer,1982 & Rossdale,1984) and the viability of the fetus is established immediately from observation of fetal movements and fetal heartbeat (Ginther,1986). More work has been reported in the mare and this includes;-

(a) Monitoring progress of conceptus within the uterus (Leith and Ginther,1984).

(b) Detection and confirmation of twin pregnancies (Simpson *et al.*,1982).

(c) Ovarian follicular dynamics (Ginther and Pierson,1984a).

(d) Cyclic changes within the uterus (Hayes *et al.*,1985).

(e) Pathological changes in the uterus such as uterine cyst and pyometra (Ginther and Pierson,1984b).

(f) Early detection of embryonic losses (Ginther *et al.*,1985).

Similarly, transrectal diagnostic ultrasonography has profoundly enhanced one's ability to visualize and evaluate the reproductive organs in cattle. It has made possible the study of the dynamic interactions within the ovarian follicular population. Pierson and Ginther (1988) reported that follicles as small as 2 to 3 mm can be visualized, quantified and sequentially monitored. Omran (1989) reported similar results. The accuracy of diagnostic ultrasonography for assessment of ovarian structures has been reported to be between 83% and 95% (Kahn and Leidl,1986 & Pierson and Ginther,1988). In contrast Huhold (1982) reported 65% accuracy with rectal palpation. Pierson and Ginther(1988) detected ovulation on the basis of disappearance of a large follicle that was present at previous examinations and subsequent formation of a luteal gland. The corpus luteum gives a different echogenic pattern than that of surrounding tissue and has a defined border on an ultrasound image (Pierson and Ginther,1984a). Omran (1989) was able to recognise the formation of corpus

haemorrhagicum on day 1 and by day 3 he was able to distinguish the corpus luteum from the surrounding ovarian stroma. Ultrasound also allows the detection of morphological changes of the uterus during the oestrous cycle by monitoring changes in shape and echotexture (Fissore *et al.*,1986). Pregnancy can be detected as early as day 9 and by day 22 it can be confirmed in 100% of cases (Boyd *et al.*,1988 & Kastelic *et al.*,1989). Omran (1989) and Curran *et al.*(1986) established that embryonic death had occurred by observing cessation of fetal movements i.e. heartbeat. As the use of this technique advanced, it became possible to diagnose pathological conditions of the uterus and the ovaries such as metritis, pyometra, macerated or mummified fetus and follicular and luteal cysts (Edmondson *et al.*,1986; Fissore *et al.*,1986 & Sprecher and Nebel,1988).

In summary, ultrasonography provides a non-invasive visual image of the reproductive tract and facilitates detection and evaluation of normal morphological changes and allows examination of pathological processes. As ultrasound gains popularity in veterinary medicine it is becoming clear that ultrasound scanners are destined for a broader, more fundamental role in bovine and equine reproduction.

As discussed above ultrasound is particularly suited to an investigation of ovarian and uterine function during the cycle after insemination and for the study of embryo viability. Some research has already been done correlating endocrine changes and ultrasonographic findings during this time (Omran,1989), and the present study was undertaken to extend this work and investigate reproductive events in cows from a commercial herd with a clinically significant repeat breeder problem.

CHAPTER TWO

MATERIALS AND METHODS

(1) THE ANIMALS

The animals used in this study were part of a commercial dairy herd at the Cochno farm of the University of Glasgow. They were kept in a covered shed with free access to an open yard during the winter and fed silage *ad libitum* and brewers grain which comprised mainly wheat and barley. In the latter part of the study all the animals were turned out to grass. The animals were milked twice daily and whilst in the parlour they were given dairy cake. They were monitored 6 times a day for oestrus activity and the majority of services were performed by A.I.. Tests carried out in 1989 suggested that leptospirosis and infectious bovine rhinotracheitis were endemic in the herd and consequently, the whole herd was vaccinated against leptospirosis in November of the same year. A total of 10 lactating cows were observed in this study, with one cow being followed twice. Animals were divided into two groups. Group one were designated as controls and were observed after first service post-partum. Group two comprised cows designated as repeat breeders i.e. they had returned to service at least three times. Animals that conceived were observed for 42 days from the service date and those that failed to conceive were only observed until the day they returned to service again. The animals were examined as they became available and not in any particular order or group. The animals were selected using the herd records which were computerised (IBM PS/2, model 30) using a Daisy programme (Department of Agriculture, University of Reading, Reading, Berkshire, U.K.) and the breeding history of the animals observed in this study is given in Table 2.1.

TABLE 2.1 Calving date and number of services per animal in cows designated as controls (Group 1) and repeat breeders (Group 2).

GROUP 1

Cow number	Calving date	Number of services
4	19/12/90	1
34	24/01/90	1
21	03/02/90	1
73	11/02/90	1

GROUP 2

Cow number	Calving date	Number of services
29	13/06/89	3
10	26/06/89	5
2	03/07/89	5
1	07/08/89	5
72	12/09/89	3
14	07/12/89	3

During the period of study, cows were observed regularly using an ultrasound and blood samples were taken for measurement of plasma progesterone concentration and records of oestrus were kept.

(2) PHYSICAL PRINCIPLES OF ULTRASONOGRAPHY.

The basic physical principles of ultrasonography and image interpretation, which have been reviewed in depth by Park *et al.*(1981), Ginther (1986), and Barr (1988), are considered before discussing the technique for transrectal ultrasonographic examination. The ultrasound transducer contains one or more crystals with piezo-electric properties. When electrically stimulated, the crystals become deformed and consequently emit sound waves of a characteristic frequency. When the transducer is placed in contact with the surface of the body, sound waves travel through the tissues. Interfaces between tissues of differing acoustic impedance reflect part or all of the beam back towards the transducer. The returning echoes are received by the same crystals and converted by means of the piezo-electric effect into electrical signals, which are analysed according to the strength and depth of reflection, and displayed on an oscilloscope screen.
There are several ways of displaying the electrical signals received ;

(i) A MODE; or Amplitude mode. This is a simple method characterised by a single line on the screen. The horizontal axis represents distance and the vertical axis represents the strength of the returning echo.

(ii) B MODE; or Brightness mode. Many scan lines are emitted sequentially by a single moving crystal or an array of crystals. A two dimensional image representing a slice through the body in the plane of the beam is built up. In this instance, the strength of the returning echo is shown by the brightness of the spot on the screen. In 'real time' scanning, the image produced is continuously updated to allow movement to be seen. Real time scanning is the most commonly used technique in medical and veterinary ultrasound.

(iii) M MODE; or Time motion. This is an adaptation of real time scanning. A cursor allows selection of one line on the B mode scan. In isolation this would be shown as a single vertical line composed of dots of varying brightness representing the interface crossed. This vertical line is however, continuously updated and the image is moved along a horizontal axis, thus showing movements of structures along that line. This technique is used only in cardiac evaluation.

Equipment.

There are two main types of transducers available;

(a) Linear array: These have crystals arranged in a line along the transducer, each producing sound waves. The sound beam thus formed is rectangular in shape, which allows superficial structures to be more readily imaged and makes it relatively easy to analyse the anatomical relationship between them.

(b) Sector: These contain a single crystal which oscillates or rotates to produce a fan shaped beam. The small size and manoeuvrability of these transducers allow ready access to most thoracic and abdominal viscera. However, superficial structures are less easily imaged due to the shape of the beam.

The overall brightness of the image can be altered by changing the power output of the transducer. This simply alters the amount of sound emitted, and consequently the amount of sound returning. Too little power results in loss of fine detail, while too much power obliterates detail due to too many echoes. The resolution and depth of penetration required determines the selection of frequency of the scanner. A higher frequency will have reduced penetration but provide better resolution, whereas, a lower frequency will penetrate further but the resolving capabilities are poor. Resolution refers to the ability of an

ultrasound pulse to distinguish between two closely spaced structures and it is divided into lateral and axial resolution. In lateral resolution, the reflectors are located at right angle to the beam and it will only be able to distinguish the two reflectors if there are separated by space greater than the width of the beam. Otherwise the two echoes arrive at the transducer at the same time and will be perceived as a single echo. In axial resolution, the reflectors are located along the direction of the beam. This means that longer pulses are more likely to overlap the two reflecting surfaces than shorter pulses. Thus high frequency transducers give better axial resolution.

Principles of image interpretation.

A number of terms are used to describe the image and some common synonyms are listed below;

(i) Hyperechoic; echogenic:- Bright echoes appearing white on conventional scans. Represent highly reflective interfaces (eg.bone and air).

(ii) Hypoechoic; relatively echolucent:- Sparse echoes, appearing dark grey on conventional scans. Represents intermediate reflection/transmission (eg. soft tissue).

(iii) Non-echogenic; anechoic; echolucent; sonolucent:- Absence of echoes, black on conventional scans. Represents complete transmissions of sound (eg. fluids).

Interpretation.

Proper interpretation of the echoes on an ultrasound screen is crucial. Interpretation of gray scale ultrasound scans depends on a knowledge of cross-sectional anatomy, scanning artefacts and basic principles. The principles of ultrasonography centre on the ability of sound waves to be either reflected from or propagated through various tissue interfaces. However, certain tissue formations also cause waves to bend (refract), bounce back and forth or re-echo (reverberate), become weakened (attenuated) or entirely blocked.

An understanding and knowledge of artefact production and identification is necessary to avoid or diminish interpretation errors. An artefact on a B mode gray scale ultrasound image has been defined as any dot appearing in the ultrasound image that does not correspond to a real echo in the patient. This definition has been broadened to include any alteration in the ultrasound image which does not portray a true representation of actual structures.

Echo distortion caused by ultrasound-matter interaction occurs more frequently than other artefacts. Artefacts of this type include:-

(i) Reverberation. These artefacts are produced by a sound pulse bouncing back and forth between two interfaces and are the most frequent and troublesome artefacts produced on ultrasound imaging. They may be produced by sound waves reflecting between the transducer and tissue interface or internally between two reflecting interfaces. Transducer interface reverberation echoes are produced each time the sound pulse returns to the transducer. The time lapse that occurs between the second, third, or fourth returning echo places them at a greater depth in tissue on the recorded image. If there is sufficient amplitude remaining after the second trip, this process may continue producing additional echo signals that are equidistant from one another but progressively weaker. The following three distinguishing features help identify reverberation artefacts;

(a) they are equidistant.

(b) they gradually diminish in intensity.

(c) they are orientated parallel to the reflective interface and appear as a column of echoes.

Reverberation artefacts are very common in the pelvic area because of pockets of bowel gas. In addition, the many fluid filled structures in the female reproductive tract increase the potential for legible reverberations.

(ii) Shadowing. A shadow is caused by a noticeable decrease or absence of ultrasonic waves due to blockage or deviation of the sound beam. In other words it is caused by complete reflection or attenuation of the sound beam. The zone deep to the reflecting or attenuating structure is anechoic; however, faint reverberation echoes may appear in the shadowed area. Shadowing may be produced by bone, gas or calculi and impede adequate imaging of deeper structures.

(iii) Refractive and reflective acoustic shadowing zones. May occur distal to margin of a rounded structure containing material of lower acoustic velocity, such as fluid filled structures. The sound penetrating the edge of such a structure may be slightly refracted or reflected producing a linear anechoic zone deep to the structure. The smooth surfaces of the uterus, embryonic vesicles and uterine cysts are all examples of this.

(iv) Enhancement or through transmission artefacts. Are caused by a relative lack of sound attenuation. These artefacts result when the ultrasound beams pass through a reflector-free structure that is fluid filled. The beam is not attenuated by echo production while passing through the fluid; that is the fluid is non-echogenic. Therefore, when the beam emerges from the far side, the amplitude of the pulse is greater than in the tissue on each side. The relatively greater amplitude or strength of sound beams distal to the fluid filled structures results in a column of relatively brighter echoes beneath the structure.

(v) Other artefacts. Ultrasound scanners are highly complex, and the images are subject to aberration due to engineering flaws or shortcuts, malfunctioning and outside electrical interference.

(3) TECHNIQUE FOR TRANSRECTAL ULTRASONOGRAPHIC EXAMINATION.

A real time, B-mode, diagnostic ultrasound scanner (Concept II Dynamic Imaging, Livingstone, Scotland) equipped with a linear array, 7.5 MHz, transducer designed for intrarectal placement was used in this study. The scanner had the following additional facilities, a freeze mode, a magnification or zoom mode, an integral electronic pen measuring device and image storage memory with recall. Furthermore, the scanner had a press control key board with annotation facilities which permitted images to be permanently identified on videotapes and photographs. To avoid any discrepancy and to maintain optimum consistency in the results the same scanner and transducer were used throughout the study. The above equipment plus a video recorder were mounted on a mobile trolley to ease movement from one animal to another. The animals were scanned once during the following days :-

(a) 4-6 days post standing oestrus.

(b) 13-15 days post standing oestrus.

(c) 19-20 days post standing oestrus.

(d) 23-25 days post standing oestrus.

(e) 29-31 days post standing oestrus.

(f) 40-41 days post standing oestrus.

All cows were inseminated at oestrus but those cows which returned to oestrus were no longer examined after that time. The technique employed for transrectal ultrasound examination in this study has been previously described by Boyd *et al.*,(1988), Pierson *et al.*,(1988) and Omran (1989). The preparation of the cow for ultrasound examination is similar to that required for manual rectal examination, that is, restraint of the animal and manual evacuation of rectal faeces. However, the restraining technique employed for ultrasound examination should be such that movement of the animal is minimised. Excessive movement of the animal interferes with the interpretation of the ultrasound image. The animals

were thus chained around the neck within the stands in the byre, and scanned. Similarly, the evacuation of the rectal faeces for ultrasound examination had to be more thorough than that required for a manual rectal examination, otherwise the faecal material adhered to the transducer and this resulted in shadowing of the ultrasound image. Before scanning, rectal palpation was carried out in order to locate the reproductive tract and familiarise the operator with the structures present. The probe was placed inside a disposable rectalling glove with coupling gel to prevent transmission of any infectious disease from one animal to another. Then the probe was carried gently into the rectum in the palm of the operator's hand and was manipulated in such a way that it lay in close proximity to the ventral rectal wall to establish good contact with the moist mucosa. The probe was then directed over the dorsal surface of the genital tract through the rectal wall. The presence of air between the acoustic face of the probe and the rectal mucosa greatly reduced the image quality and this too was eliminated by using coupling gel. However, manipulation of the reproductive tract before hand was often required in order to obtain a clear image. After the initial orientation the examination of the genital tract was carried out in a defined sequence as follows:-

(a) right ovary.

(b) left ovary.

(c) right horn.

(d) left horn.

(e) cervix.

Using the light pen electronic caliper directly on to the frozen screen image of the scanner the following measurements were taken;-

(a) height of the follicles present on the respective ovaries.

(b) height, width and area of the corpus luteum.

(c) dorso-ventral diameter of the uterine horn.

(d) thickness of the myometrium.

(e) single and double dorso-ventral diameter of the endometrium.

(f) size of uterine lumen.

(g) dorso-ventral diameter of cervix.

(h) size of cervical lumen.

(i) if there was a conceptus present than the linear dimension of its ultrasound image.

To locate the ovaries the probe had to be moved laterally over the dorsal surface of the reproductive tract. Follicles, like other fluid filled structures, appeared on the ultrasound image as black (non-echogenic) areas (Fig. 9a and 9b). The follicles were roughly circumscribed and irregular shapes were attributable to compression between adjacent follicles or between a follicle and a luteal structure or ovarian stroma. The measurement recorded as the follicular diameter was actually antral diameter because the border between antrum and follicular wall was much more distinct than between the follicular wall and stroma. However, care was required to minimise confusing follicles with other non-echogenic areas, such as the non-luteinised central portion of a corpus luteum or cross sections of a blood vessel. Follicles could usually be distinguished from other non-echogenic areas by a defined, relatively smooth outline.

Unlike the follicle the corpus luteum has a well defined border and different echotexture from that of the ovarian stroma. This allowed the measurement of the corpus luteum within its defined outline in the ovarian stroma. Some luteal glands contained centrally located, fluid filled areas (lacunae) and these too were measured and recorded (Fig. 9c).

The ultrasonographic appearance of the uterus depends on the stage of the oestrous cycle. The thickness of the myometrium was taken from the dorsal border of the uterine horn to the dorsal border of the endometrium (Fig. 9d). The single endometrium thickness was measured from the dorsal border with the myometrium to the uterine lumen. The measurement from the dorsal border with the myometrium to the ventral border with the myometrium gave the double endometrium thickness (Fig.9d). The presence of a lumen was detected as a non-echogenic (dark) area on the screen. The cervix being a dense mass of tissue gave a highly echogenic appearance and its total thickness (dorso-ventral diameter) was measured (Fig. 9f). However, when the cervix was laid over the pelvis, difficulty arose in distinguishing the ventral border of it from the dorsal border of the pelvis since both gave a hyperechogenic image on the scanner.

To detect early pregnancy the transducer had to be guided cranially towards the confluence of the uterine horn with the uterine tubes and the first day of detection of a discrete non-echogenic structure in the uterine lumen was recorded as the first day of presumptive detection of an embryonic vesicle. A confirmed pregnancy was defined as subsequent progressive elongation of the non-echogenic area within the uterus proper (Fig. 9e). The embryo proper was defined as a distinct echogenic spot within the non-echogenic vesicle. Confirmation of the presence of an embryo proper was based on the detection of a heart beat. Once the elongating vesicle was located, it was measured as was the length of embryo proper when it subsequently appeared. However, in pluriparous cows it was difficult for the operator to reach the confluence of the uterine horn and the fallopian tubes especially when the uterus lay cranial to the pelvic brim and into the abdominal cavity.

Finally, all ultrasound scanning was recorded on videotapes and later played back on a high quality viewer. The viewer had slow motion as well as freeze frame facility, which enabled frames of particular interest to be followed sequentially. In addition, this facility helped in clarifying doubts which rose whilst scanning the cows on the farm. A thermal printer (Sony UP811) was connected to the viewer and prints of selected frames were recorded onto sensitised paper. For sake of permanent documentation the thermal prints were photographed.

(4) BLOOD SAMPLING PROCEDURE.

A single blood sample (5ml) was collected from all animals from the median caudal (coccygeal) vein using evacuated heparinised tubes and 20G x 1 inch needles (Vacutainer Systems, Becton and Dickson). The blood was collected from the animals on the following days with respect to oestrus:-

(a) day 0-day 14, every 2-3 days.

(b) day 14- day 24, everyday.

(c) day 24-day 41, every 2-3 days.

The plasma was harvested by centrifuging the blood at 3000 rpm for 10 minutes and was stored at -20 C in the deep freezer until it was assayed.

(5) RADIOIMMUNOASSAY OF PLASMA PROGESTERONE.

Plasma progesterone concentrations were determined by duplicate diethyl extracts of both sample and standards (0-15ng/ml). All reagents were diluted using 0.05M phosphate buffered saline containing 0.25% bovine serum albumin (BSA). The first antibody was provided by The Royal Infirmary, Glasgow, and was raised in sheep against 11a-hydroxy-progesterone hemisuccinate BSA and used at an initial dilution of 1:15,000 to give approximately 30-35% binding of labelled progesterone. Cross reactions of this antiserum with 11a-hydroxprogesterone, 11-deoxycorticosterone, 17a-hydroxyprogesterone and 20a-hydroxypreg-4-en-3-one

were 61%, 4%, 5%, and 1%, respectively and <1% with all other steroids tested. The plasma samples and the standards were extracted with 3ml of ether (Analar grade, May and Baker, Dagenham, U.K.) by vortex mixing for approximately 5 minutes on a multivortexer (Alpha Laboratories, Eastleigh). The ether phase was decanted into glass tubes after prior freezing of the aqueous phase in a bath of methanol and dry ice, and evaporated to dryness under air. Iodinated progesterone was provided by The Royal Infirmary, Glasgow, and was prepared using progesterone 11a-glucuronyl tyramine, which was iodinated using chloramine T, and purified by solvent extraction and thin layer chromatography. Tracer (100ul aproximately 10,000 cpm) and primary antibody (200ul) were added to the dried ether extracts and incubated at 37 C for at least 45 minutes. Then 400ul of second antibody containing 1:15 donkey anti-sheep/goat serum plus 1:150 normal goat serum (Scottish Antibody Production Unit, Law hospital, Carluke, Lanarkshire, Scotland) was added and tubes were incubated at 4 C overnight. The following day the tubes were centrifuged at 2,000 g for 10 minutes. The supernatant was aspirated and discarded while the precipitate containing the bound tracer was counted in a Packard Auto-Gamma Minaxi 5000 series counter equipped with a complete data reduction facility. Counts were automatically corrected for non-specific binding and duplicates were averaged. Log hormone concentration was plotted on the X-axis against counts bound divided by total counts bound x 100% on the Y-axis. The assay sensitivity was >0.1 ng/ml. The intra-assay coefficient of variation for two pools containing mean progesterone concentration of 0.76 ng/ml and 2.9 ng/ml were 5% and 10% respectively. The inter assay coefficient of variation for two pools containing mean progesterone concentrations of 2.8 ng/ml and 6.3 ng/ml were 10% and 11% respectively.

CHAPTER THREE

RESULTS

The results from ultrasonic observations and measurement of plasma progesterone concentration for the cows in the control group were very similar and have thus been pooled together and presented as a group. However, the results obtained from cows in the repeat breeder group varied from animal to animal and so are presented individually.

Group 1 (control group)

All four cows in the control group held to the first service *post partum* and were still pregnant on the last day of scanning (i.e. day 42) as shown in a summary of reproductive histories in Table 3.1. The number of days open (calving to conception) ranged from 75 to 91 (Table 3.1).

(i) Ultrasonic observation of the ovary and uterus. In all the control cows, both ovaries were active throughout the period of study and the height of the largest follicle present on each ovary is shown in Figure 1c. The ovary contralateral to the corpus luteum bearing ovary showed more follicular activity with follicles ranging from 2-14mm in height whereas the height of the largest follicle present on the corpus luteum bearing ovary was not greater than 10mm. The corpus luteum by day 4-6 after service had a different echotexture and a well defined outline which made it distinct from the surrounding ovarian stroma and easily recognisable as seen in Figure 10a and 11a. In addition, a lacuna could be seen on the ultrasonic image as an irregularly shaped non-echogenic area within the centre of the corpus luteum. The corpora lutea in the control cows had increased to maximum size by day 13-15 and possessed a heterogeneous echotexture as depicted in Figure 10b. By day 19-21 the corpus luteum showed a slight reduction in size but maintained the

heterogeneous echotexture seen in the mid-cycle corpus luteum (Fig. 10c). From days 25 to 42 little variation in the echotexture of the corpus luteum was observed (Fig. 10d, 10e and 10f) but the height, width and area of the corpus luteum fluctuated throughout this period (Fig. 1a and 1b). In cow 34 two corpora lutea were observed, one on each ovary. The one on the left ovary (ipsilateral to the pregnant horn) was maintained whereas the one on the right ovary decreased in dimension as well as in echogenicity and its outline became obscure by day 24 as depicted in Figure 11d. These signs of regression continued and follicles of varying sizes appeared on the ovary.

Profound morphological changes were observed within the uterus using ultrasound as shown in Figure 12 and 13. An embryonic vesicle was detected in all four animals by day 15-18 and appeared as a non-echogenic area in the uterine horn ipsilateral to the corpus luteum (Fig. 12b and 12c). The vesicle increased in size and by day 25 it contained an echogenic structure which was the embryo (Fig. 12d). In cow 34 and 73 embryonic heartbeat was detected as a pulsatile movement emanating from the rod like echogenic structure within the vesicle. By day 42 the embryo was clearly visible and the outline of it became well defined with different regions of the embryo now discernable (Fig. 12f).

(ii) Plasma progesterone concentration. Plasma progesterone concentrations in the four control cows during the period of observation are shown in Figure 1a. The plasma progesterone concentration increased steadily from a low concentration (<0.5ng/ml) on day 2 to approximately 4ng/ml by day 10. A further increment in plasma progesterone concentration (>10ng/ml) was observed in all four cows up to day 16-18. In the following 2-3 days the plasma progesterone concentration decreased in all the four cows but this was temporary and levels increased from day 22 onwards and this elevated plasma progesterone concentration was maintained for the rest of period of observation in the four cows.

Group 2 (Repeat breeders)

The reproductive histories of the repeat breeder cows are summarised in Table 3.1. Two cows in this group conceived after the third service. Studies on cow 10 were commenced after the fifth service and it conceived after being dropped from the study. The other three cows in this group were still open at the end of the study. The number of services per animal in this group varied from 3 to 5 and days from calving to first service ranged from 59 to 134 days. The number of days open ranged from 177 to 224 days in the cows that conceived.

COW 29.

(i) Ultrasonic observation of the ovary and uterus. Cow 29 conceived to the third service and was still pregnant when scanned on day 63 post-insemination. Ultrasonographically the echotexture of the corpus luteum did not vary from that seen in control cows throughout the observation period (Fig. 15). Measurements of the height, width and area of the corpus luteum are shown in Figure 2a and 2b. These fluctuated throughout the observation period and values were similar to those described for the control cows. Both ovaries showed follicular activity and follicles attained much higher dimensions on the contralateral ovary compared to the corpus luteum bearing ovary (Fig. 2c and Fig. 14).

As in the control cows, the presence of a non-echogenic embryonic vesicle was detected by day 14 and was seen to have increased in size at subsequent examinations (Fig. 16b and 16c). The embryo proper was identified by day 24 as an echogenic structure within the non-echogenic vesicle. It increased in size and by day 42 was similar in all aspects to embryos imaged in control cows (Fig. 16e and

16f). This cow was also scanned on day 63 post-service and by this time the different regions of the fetus were distinguishable as seen in Figure 17a. The cotyledons were clearly seen protruding from the endometrial surface (Fig. 17b).
(ii) Plasma progesterone concentration. Changes in plasma progesterone concentration followed a similar trend to those described in the control group with low plasma progesterone concentration seen on day 2 (<0.5ng/ml), increasing gradually to a peak concentration at day 17 (Fig. 2a). Again there was a temporary decrease in the plasma progesterone concentration around day 20-22 and then an increase around day 24. Progesterone levels remained elevated thereafter to the end of the period of observation.

COW 10 AND COW 14.

(i) Ultrasonic observation of the ovary and uterus. Cows 10 and 14 returned to service on day 23 and 20 respectively, and were re-inseminated. In both cows, the corpora lutea had similar ultrasonographic characteristics as described for the control group up to day 15, as can be seen in Figure 18b. However, by day 20 in cow 10 and day 18 in cow 14, there was a reduction in the size of the corpus luteum with decreased echogenicity and more brightly imaging tissue appearing in the centre of the corpus luteum, and blurring of the outline of the corpus luteum which made it difficult to distinguish from the surrounding ovarian stroma (Fig. 18c).
By this time the height, width and area of the corpus luteum in both cows were reduced (Fig. 3a, 3b, 4a and 4b). The left and right ovary showed pronounced activity in both cows with follicles ranging in height from 7-14mm in cow 10 and from 3-14mm in cow 14 (Fig. 3c, 4c and 18).

The echotexture of the uterus was similar to that seen in control cows 5 days after insemination (Fig. 19a and 19d). However, by day 15 post service there was a failure to detect an embryonic vesicle and the myometrium could be

differentiated from the endometrium. There was an absence of endometrial folding and no intra-uterine fluid was in evidence at this time (Fig. 19b and 19e). As the next oestrus approached the endometrial folds were discernable as echogenic structures and the uterine lumen was found to be non-echogenic due to its increased fluid content (Fig. 19c and and 19f).

(ii) Plasma progesterone concentration. Both animals displayed different plasma progesterone patterns during the period of observation as depicted in Figure 3a & Figure 4a. Cow 10 exhibited a rapid rise in plasma progesterone concentration and then steady levels from day 12 to 17. Progesterone concentration increased again to a peak around day 18. The progesterone level increased more slowly in cow 14 and was only 2ng/ml by day 10. There then occurred a sudden upsurge in plasma progesterone concentration between day 10 and day 14. However, the plasma progesterone concentration started declining gradually from day 15 in cow 14 and day 18 in cow 10 and reached a minimum concentration on day 19 in cow 14 and day 23 in cow 10.

COW 2.

(i) Ultrasonic observation of the ovary and uterus. This cow was not detected as being on heat at all during the study period. The echogenic pattern of the corpus luteum had similar characteristics to that of the control cows and of cows 10 and 14 up to day 15 (Fig. 20b). In addition, the degenerative changes which were apparent in the echotexture of the corpus luteum by day 20 in cows 10 and 14 were also visible in cow 2 by day 18 and continued up to and including day 24 (Fig. 20d). The cross sectional area of the corpus luteum was smaller than that observed in control cows up to day 15 and then decreased further until day 24 (Fig. 5a). Follicular activity was apparent on both ovaries and large follicles developed from day 10. Figure 5c and Figure 20d indicate that a dominant follicle was

present on the ovaries on day 24. The follicle seen on day 24 on the right ovary was not seen in the subsequent scanning performed on day 30 and instead a new corpus luteum was detected on the ovary. The previous corpus luteum on the left ovary had regressed completely (Fig. 20e and 21e).

The uterine horns showed a homogeneous endometrium and an absence of both endometrial folds and intra-uterine fluid. This situation was still evident on day 18 post insemination (Fig. 22c and 23c). By day 24 the endometrium had become thickened with endometrial folds evident and the lumen of the uterus contained non-echogenic fluid (Fig. 22d and 23d). Although a small lumen was evident in the uterus on day 28, the endometrial folds were no longer discernable and there was a marked reduction in the heterogeneous echotexture of the endometrial wall (Fig. 22e and 23e).

(ii) Plasma progesterone concentration. The plasma progesterone concentration increased rapidly with time from a low concentration (0.2ng/ml) on day 2 to approximately 8ng/ml on day 10 (Fig.5a). Progesterone levels varied between 5 and 9ng/ml until day 22 and then declined rapidly reaching a minimum concentration at day 25. Subsequently, an increased plasma progesterone concentration was evident from day 30 reaching a peak concentration by day 42 (Fig.5a).

COW 72.

This animal was observed over two cycles. Cow 72 conceived to the first service of the experiment but later lost the conceptus and returned to service about day 45. It was re-inseminated and this time held to the service.

(i) Ultrasonic observation of the ovary and uterus. The echotexture of the corpus luteum of the first cycle will be described first. Up to day 22 the corpus luteum had a similar echotexture as seen in the control cows and cow 29 with a fairly large

centrally placed lacuna visible (Fig. 25d). However, on day 30 of scanning, the outline of the corpus luteum was still visible but a slight reduction in the heterogeneous echotexture of the corpus luteum was evident (Fig. 25e). By day 41 the corpus luteum was reduced in size and echogenicity and its outline had become vague, indicating that the process of regression of the corpus luteum was well advanced as seen in Figure 25f. The area of the corpus luteum showed a linear regression with time from day 20 post insemination as seen in Figure 6a. The height and width of the corpus luteum also regressed linearly with time and were minimal just before next oestrus (Fig. 6b). The ovaries were active throughout the period of observation and by day 41 a dominant follicle was visible on the right ovary (Fig. 6c and 25).

The embryonic vesicle was detected as early as day 13 (Fig. 27b) and by day 20 the embryo proper was identified as an echogenic structure within the non-echogenic vesicle (Fig. 27c). However, by day 30, the embryonic vesicle had a snow-like appearance in the lumen of the uterus and the embryo was no longer clearly identifiable (Fig. 27e). The lumen of the uterus was reduced in size by day 41 and the endometrial folds were evident (Fig. 26f and 27f).

In the second cycle studied in cow 72, the echotexture of the corpus luteum was similar to that described for the control group and this time no regressive changes in the echotexture of the corpus luteum were apparent upto the last day of scanning i.e. day 41 (Fig. 28). Unlike the previous cycle the area of the corpus luteum increased from day 20 onwards as seen in Figure 7a. The height and width of the corpus luteum followed a similar trend (Fig. 7b). Both ovaries indicated follicular activity and the dimensions of follicles were much greater on the contra-lateral ovary compared to the corpus luteum bearing ovary (Fig. 7c). The embryonic vesicle was detected by day 15 and the embryo proper was identified by

day 25 (Fig. 29b, 29c and 29d). It increased in size with successive scanning and by day 41 had attained similar characteristics to those described for the control group and seen in Figure 29f.

(ii) Plasma progesterone concentration. In the first cycle the plasma progesterone concentration rose steadily with time reaching 2ng/ml on day 10 and peaking at 12ng/ml on day 22. However, a sudden sharp decline in progesterone concentration to less than 0.5ng/ml was seen on day 24 (Fig.6a).

The plasma progesterone concentration in the second cycle increased with time from basal levels on day 2 to approximately 9ng/ml on day 14 and around 12ng/ml on day 20-22. After day 22, the plasma progesterone concentration remained elevated but variable for the remaining period of observation (Fig.7a).

COW 1.

This animal conceived but later lost the conceptus and developed a luteal cyst.

(i) Ultrasonic observation of the ovary and corpus luteum. The corpus luteum was recognisable with a central lacuna as early as day 6 and the lacuna was still evident in the mid-cycle corpus luteum. The lacuna reduced in size by day 20 but the corpus luteum still maintained a heterogenous echotexture (Fig. 30a, 30b and 30c). Unlike the case in control cows, the corpus luteum of this animal started to show signs of regression i.e. blurring of its borderline and decreased echogenicity by day 25 (Fig. 30d). Further degenerative changes in the echotexture of the corpus luteum were evident by day 30 with the borderline being no longer discernable as seen in Figure 30e. In addition a developing follicle was visible in the vicinity of the regressing corpus luteum (Fig. 30e). On day 41 a structure comprising a non-echogenic area surrounded by a thick band of echogenic tissue was seen on the

scanner and this was identified as a luteal cyst (Fig. 30f) approximately 45mm in diameter. The area of the corpus luteum regressed linearly until day 30. By day 40 the structure was classified as a luteal cyst and had increased considerably in size (Fig. 8a). The height and width of the corpus luteum changed in a way similar to that as described for the area of corpus luteum i.e. linear regression with time until day 30 and then a sudden increase by day 40 (Fig. 8b). The follicular activity recorded on the ovaries is shown in Figure 8c and it is evident that there was minimal follicular activity when the cystic structure was present. The cow was treated with prostaglandin on day 43 (Estrumate, Coopers Animal Health Limited, Crewe, U.K. 2ml, 500μg, i.m.). Five days after treatment, the cystic structure had reduced in size and a developing follicle was visible close by (Fig. 33b).

An embryonic vesicle was identified by day 15 which subsequently increased in size by day 25 (Fig. 32b). However, an embryo proper was not identified at all in this animal. A reduction in size of the vesicle was evident by day 30 and by day 40 the uterus had reduced in dimension and the lumen was no longer detectable (Fig. 32e and 32f). Five days after prostaglandin treatment the endometrial folds became visible and the presence of intra-uterine fluid could be seen as a non-echogenic area on the ultrasound scanner (Fig. 33c and 33d).

(ii) Plasma progesterone concentration. The plasma progesterone concentration increased steadily with time from the low concentrations seen on day 3 and reached a peak concentration around day 12. A subsequent decrease in progesterone concentration was seen between days 14 and 18 before reaching another peak by day 20. A high plasma progesterone level was maintained with only minor fluctuations up to day 26 after which there was a steep decline in plasma

progesterone concentration to basal levels by day 28. By the time that the cystic structure was detected on day 41, the plasma progesterone concentration had once again reached elevated levels (Fig.8a).

Table 3.1. Reproductive histories of cows designated as controls (Group 1) and repeat breeders (Group 2).

Group 1

Cow No.	Calving Date	Calving to 1st Service	No. of Services	Calving to Conception
4	19/12/90	76 days	1	76 days
34	24/1/90	91 "	1	91 "
21	3/2/90	77 "	1	77 "
73	11/2/90	75 "	1	75 "

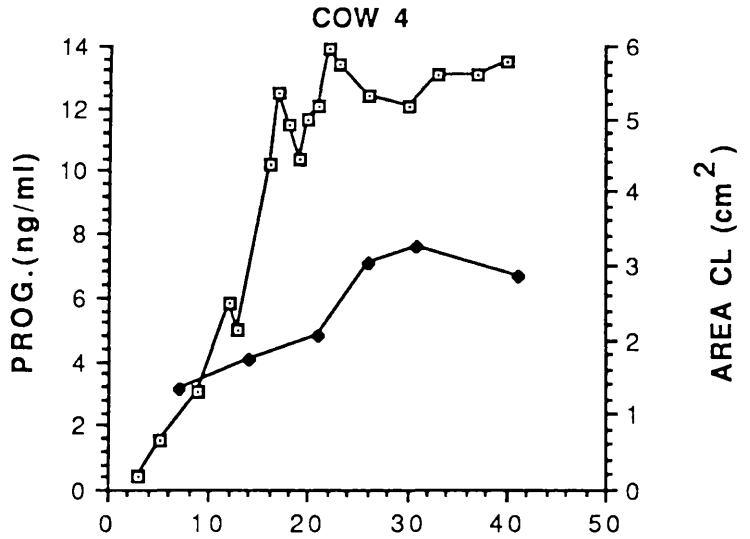
Group 2

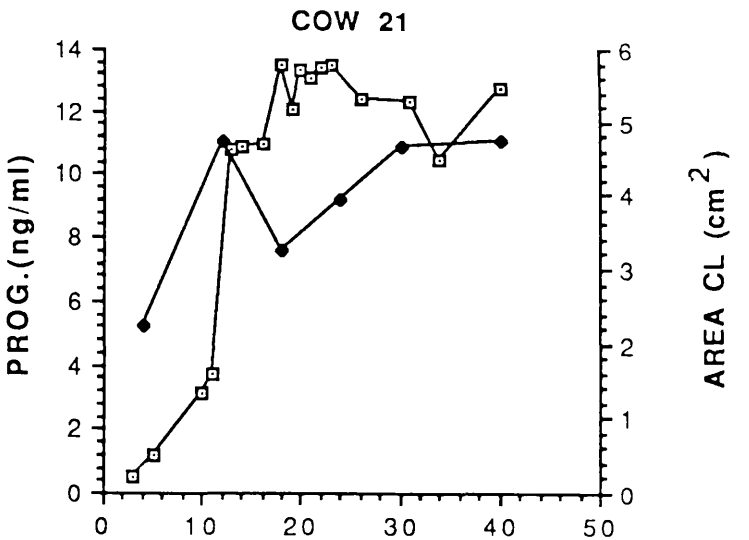
Cow No.	Calving Date	Calving to 1st Service	No. of Services*	Calving to Conception
29	13/6/89	79 days	3	211 days
10	26/6/89	63 "	5	224 "
2	3/7/89	59 "	5	
1	7/8/89	94 "	5	
72	12/9/89	134 "	3	177 "
14	7/12/89	88 "	2	

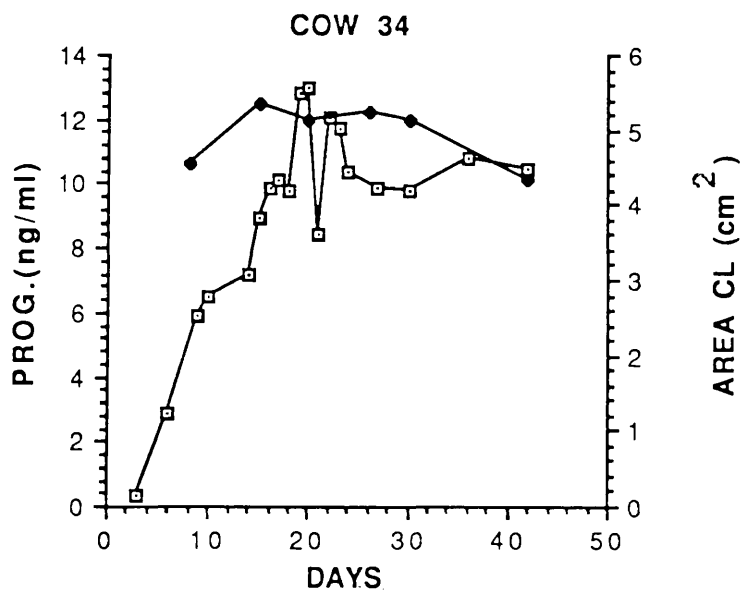
* Number of services up to and including the studied cycle

Figure 1a. Changes in plasma progesterone concentration (⊣□⊢) and corpus luteum area assessed by ultrasound (⊣◆⊢) in the four cows in group 1 over a period of approximately 40 days from day of insemination (day 0).

(a)


COW 4
PROG.(ng/ml)
AREA CL (cm^2)


COW 21
PROG.(ng/ml)
AREA CL (cm^2)


COW 34
PROG.(ng/ml)
AREA CL (cm^2)
DAYS

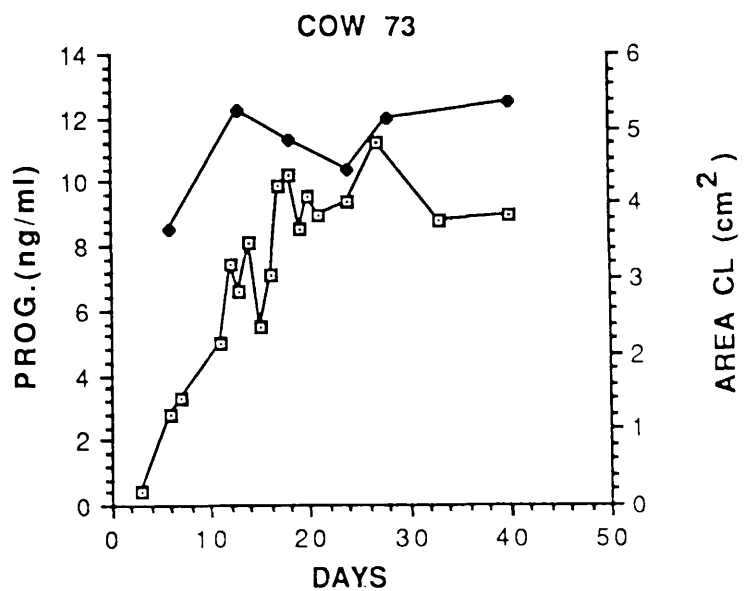
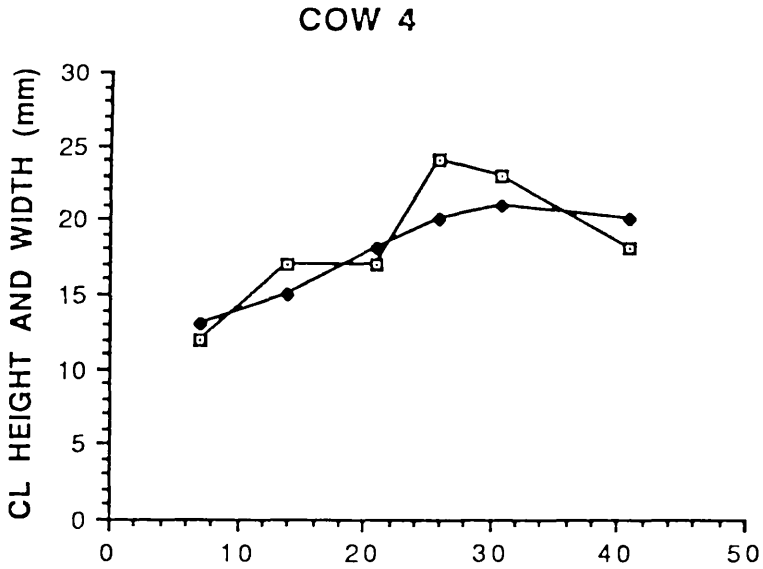
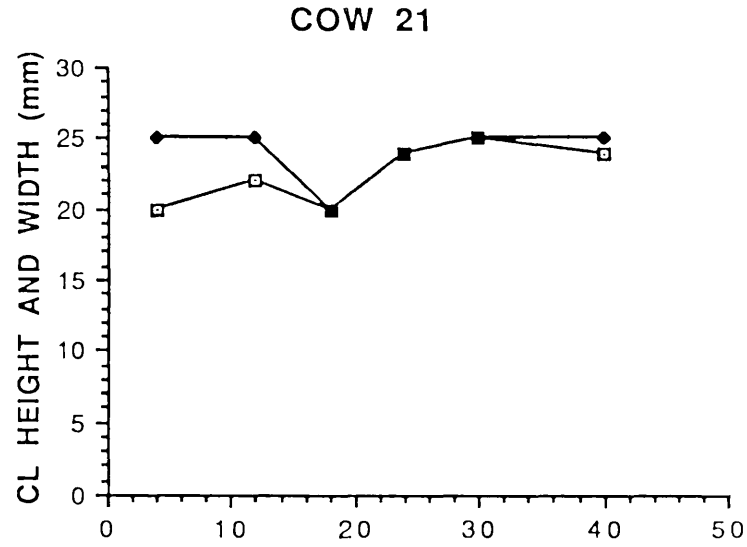
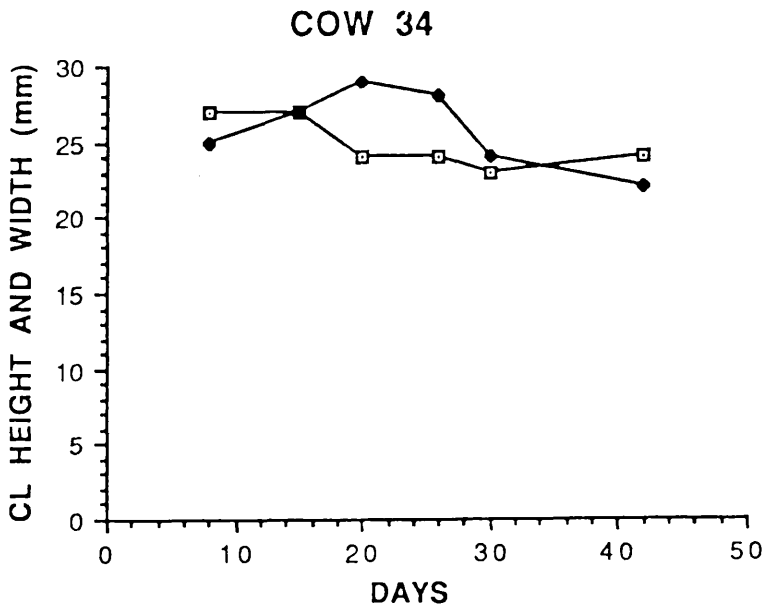

COW 73
PROG.(ng/ml)
AREA CL (cm^2)
DAYS

Figure 1b. Changes in corpus luteum height (⊢□⊣) and width (⊢◆⊣) assessed by ultrasound in the four cows in group 1 over a period of approximately 40 days from day of insemination (day 0).

(b)


COW 4
CL HEIGHT AND WIDTH (mm)
0
5
10
15
20
25
30
0
10
20
30
40
50


COW 21
CL HEIGHT AND WIDTH (mm)
0
5
10
15
20
25
30
0
10
20
30
40
50


COW 34
CL HEIGHT AND WIDTH (mm)
0
5
10
15
20
25
30
0
10
20
30
40
50
DAYS

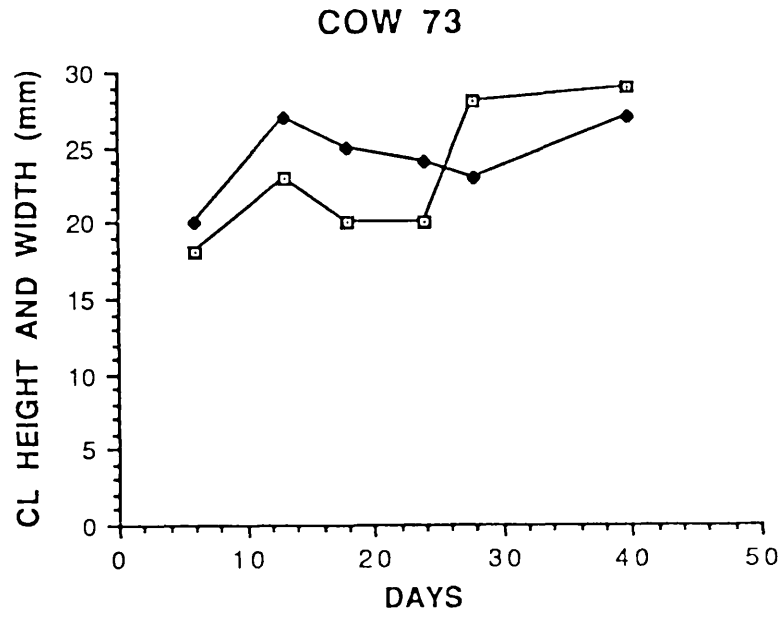
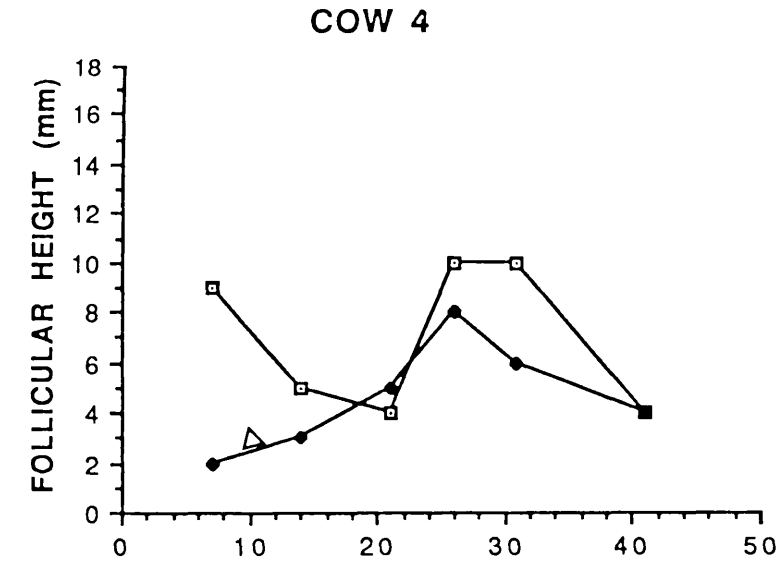
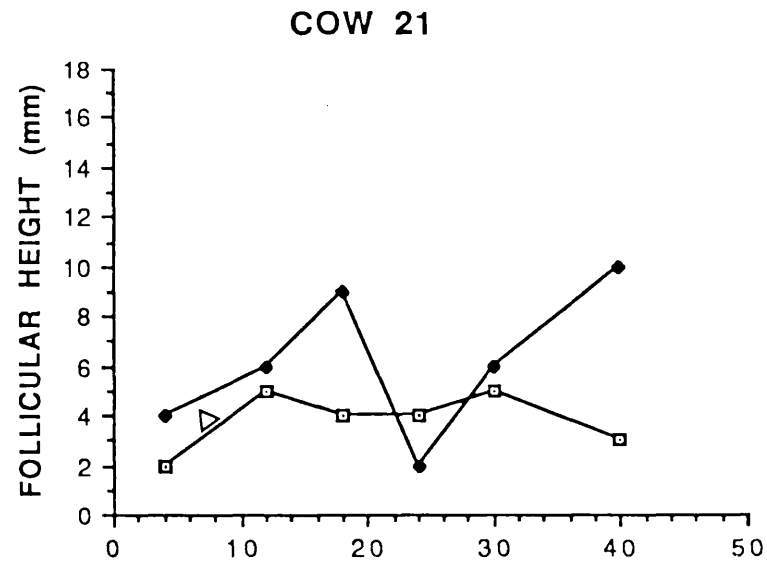
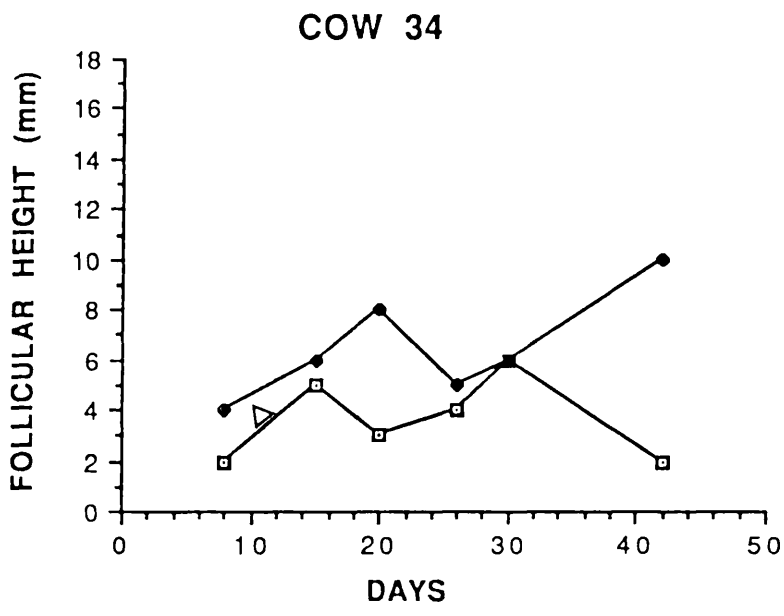

COW 73
CL HEIGHT AND WIDTH (mm)
0
5
10
15
20
25
30
0
10
20
30
40
50
DAYS

Figure 1c. Changes in height of the largest follicle detected by ultrasound on the left (⊣□⊢) and right (⊣◆⊢) ovaries of the four cows in group 1 over a period of approximately 40 days from day of insemination (day 0). (Δ) corpus luteum bearing ovary.

(c)


COW 4
FOLLICULAR HEIGHT (mm)
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50


COW 21
FOLLICULAR HEIGHT (mm)
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50


COW 34
FOLLICULAR HEIGHT (mm)
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50
DAYS

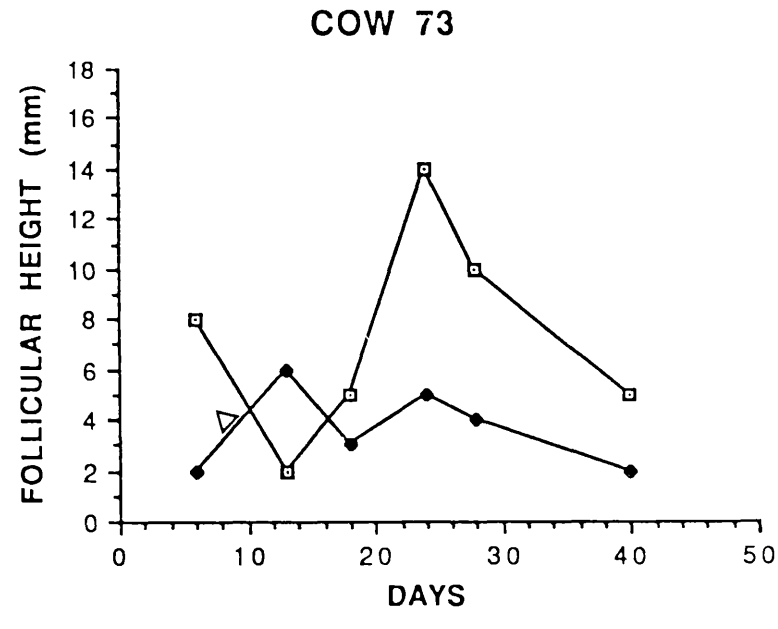
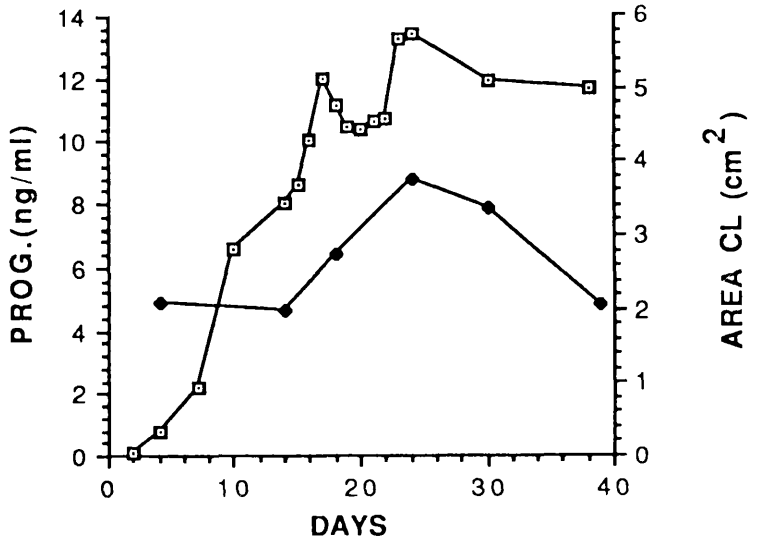

COW 73
FOLLICULAR HEIGHT (mm)
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50
DAYS

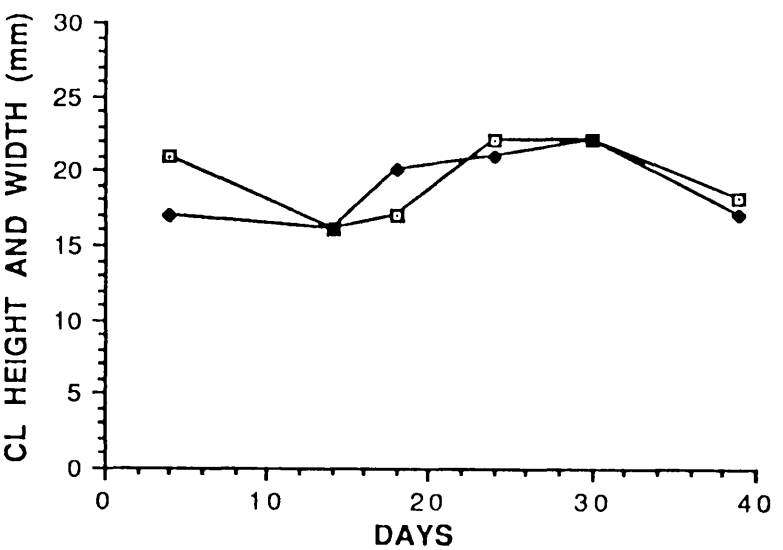
Figure 2. Changes in plasma progesterone concentration (⊣□⊢) and corpus luteum area (⊣◆⊢) **(panel a)**, corpus luteum height (⊣□⊢) and width (⊣◆⊢) **(panel b)**, maximum follicle height on the left (⊣□⊢) and right (⊣◆⊢) ovaries **(panel c)**, in cow 29 (Group 2) over a period of approximately 40 days from day of insemination (day 0). (Δ) corpus luteum bearing ovary.

COW 29

(a)


PROG.(ng/ml)
AREA CL (cm^2)
DAYS
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
0
10
20
30
40

(b)


CL HEIGHT AND WIDTH (mm)
DAYS
0
5
10
15
20
25
30
0
10
20
30
40

(c)

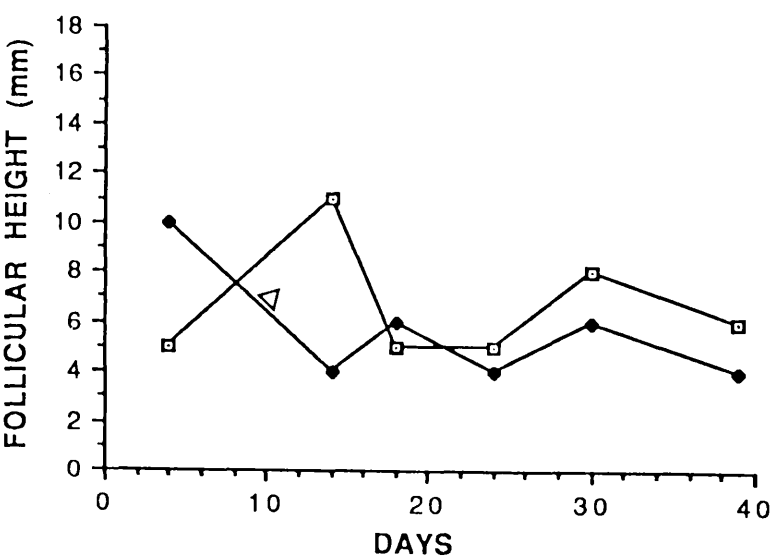
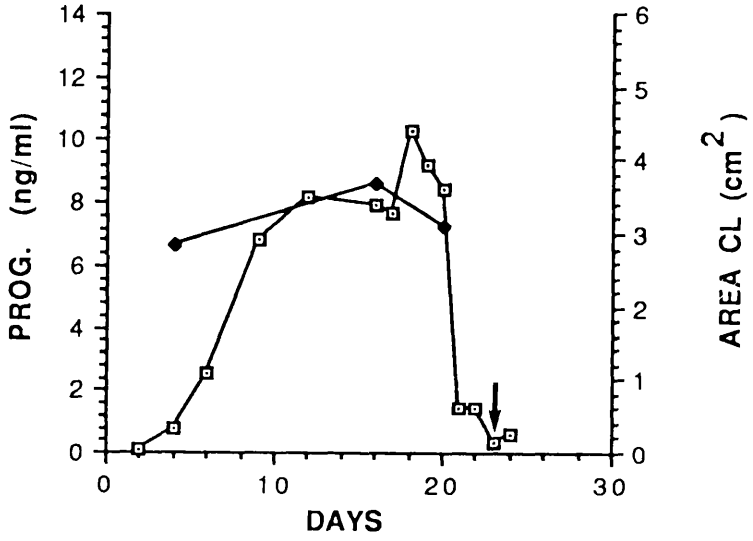

FOLLICULAR HEIGHT (mm)
DAYS
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40

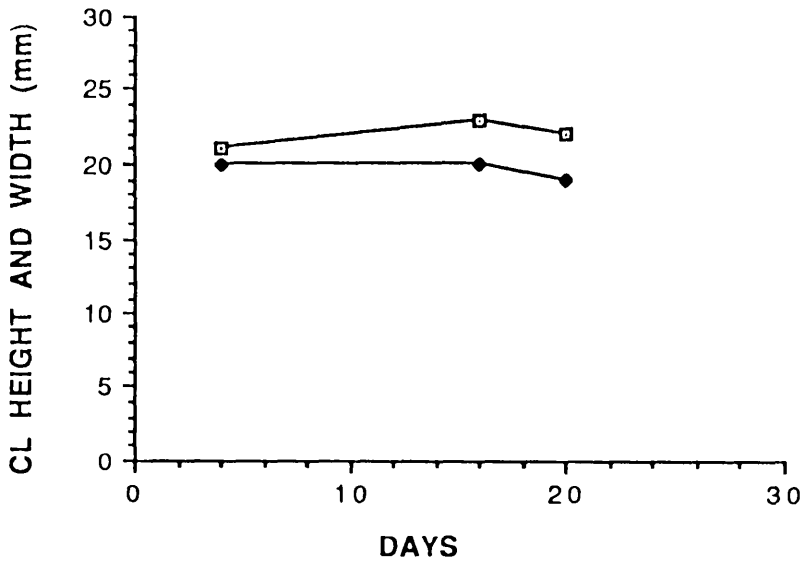
Figure 3. Changes in plasma progesterone concentration (–⊡–) and corpus luteum area (–◆–) **(panel a)**, corpus luteum height (–⊡–) and width (–◆–) **(panel b)** and maximum follicle height on the left (–⊡–) and right (–◆–) ovaries **(panel c)** in cow 10 (Group 2) over a period of approximately 25 days from day of insemination (day 0). (↓) day of oestrus. (Δ) corpus luteum bearing ovary.

COW 10

(a)


PROG. (ng/ml)
AREA CL (cm^2)
DAYS
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
0
10
20
30

(b)


CL HEIGHT AND WIDTH (mm)
DAYS
0
5
10
15
20
25
30
0
10
20
30

(c)

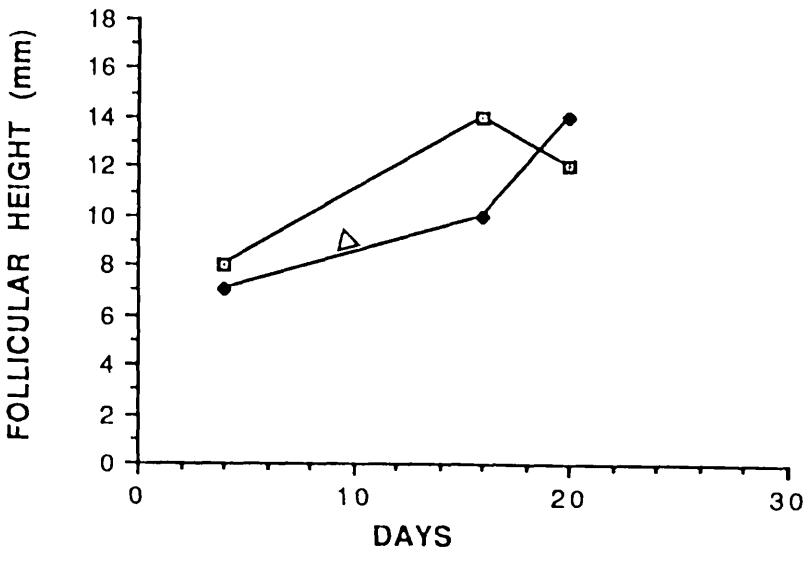
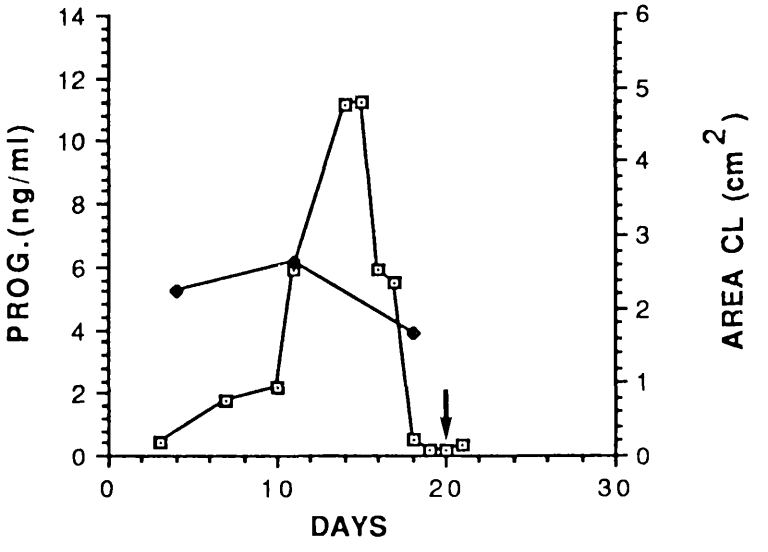

FOLLICULAR HEIGHT (mm)
DAYS
0
2
4
6
8
10
12
14
16
18
0
10
20
30

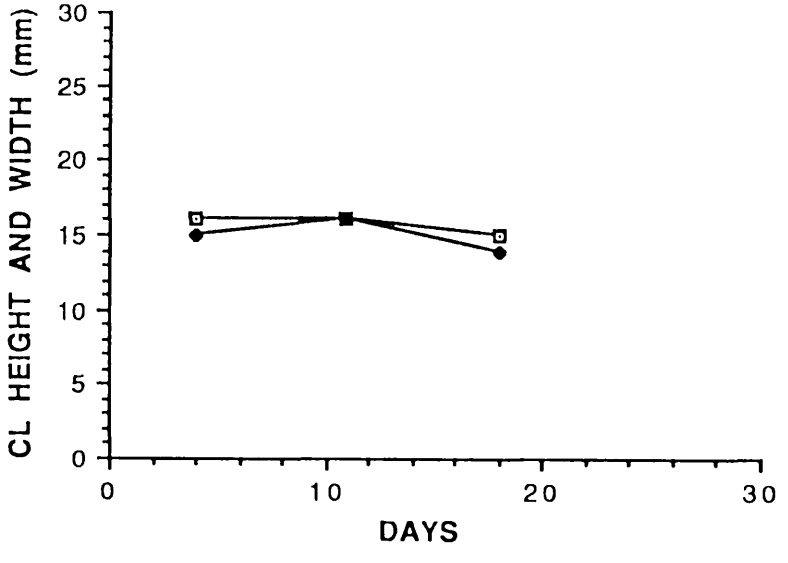
Figure 4. Changes in plasma progesterone concentration (⊣□⊢) and corpus luteum area (⊣◆⊢) **(panel a),** corpus luteum height (⊣□⊢) and width (⊣◆⊢) **(panel b)** and maximum follicle height on the left (⊣□⊢) and right (⊣◆⊢) ovaries **(panel c)** in cow 14 (Group 2) over a period of approximately 22 days from day of insemination (day 0). (↓) day of oestrus.(Δ) corpus luteum bearing ovary.

COW 14

(a)


PROG.(ng/ml)
AREA CL (cm 2)
DAYS
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
0
10
20
30

(b)


CL HEIGHT AND WIDTH (mm)
DAYS
0
5
10
15
20
25
30
0
10
20
30

(c)

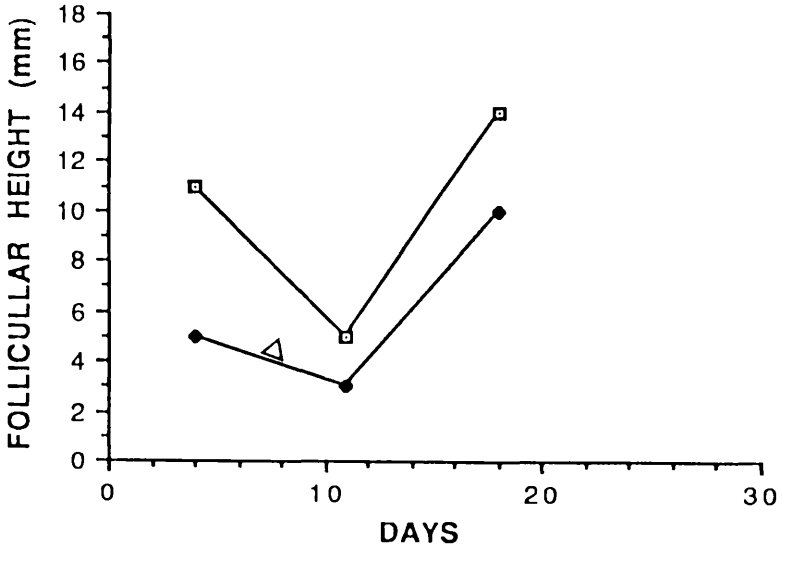
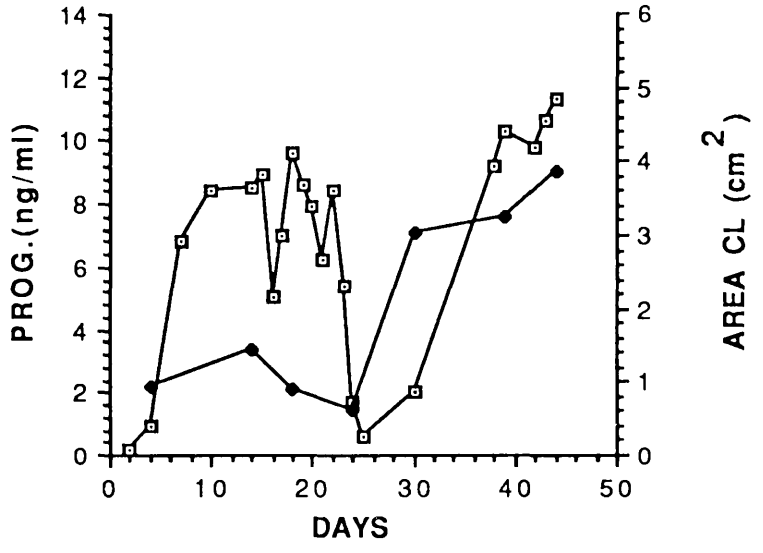

FOLLICULLAR HEIGHT (mm)
DAYS
0
2
4
6
8
10
12
14
16
18
0
10
20
30

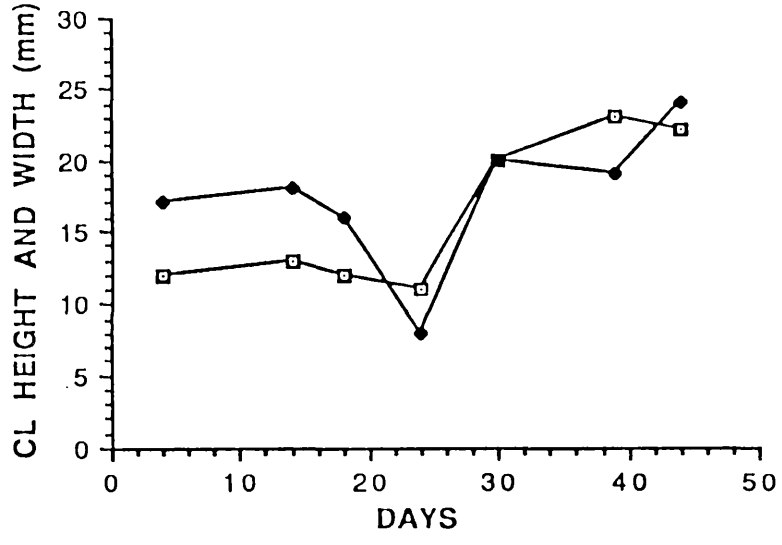
Figure 5. Changes in plasma progesterone concentration (⊣□⊢) and corpus luteum area (⊣◆⊢) **(panel a)**, corpus luteum height (⊣□⊢) and width (⊣◆⊢) **(panel b)** and maximum follicle height on the left (⊣□⊢) and right (⊣◆⊢) ovaries **(panel c)** in cow 2 (Group 2) over a period of approximately 45 days from day of insemination (day 0). (Δ) corpus luteum bearing ovary.

COW 2

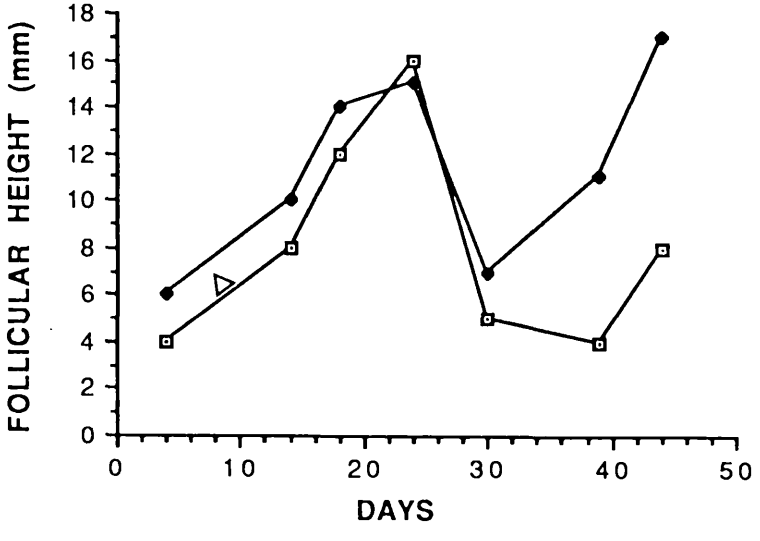
(a)


PROG.(ng/ml)
AREA CL (cm 2)
DAYS
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
0
10
20
30
40
50

(b)

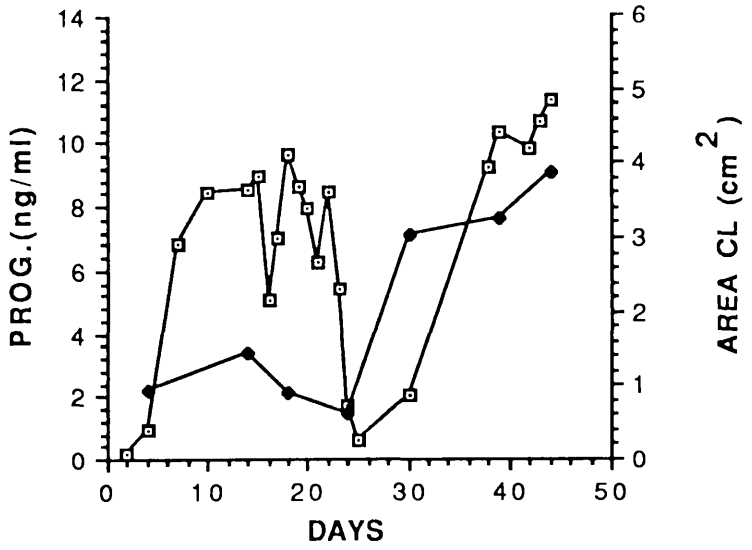

CL HEIGHT AND WIDTH (mm)
DAYS
0
5
10
15
20
25
30
0
10
20
30
40
50

(c)

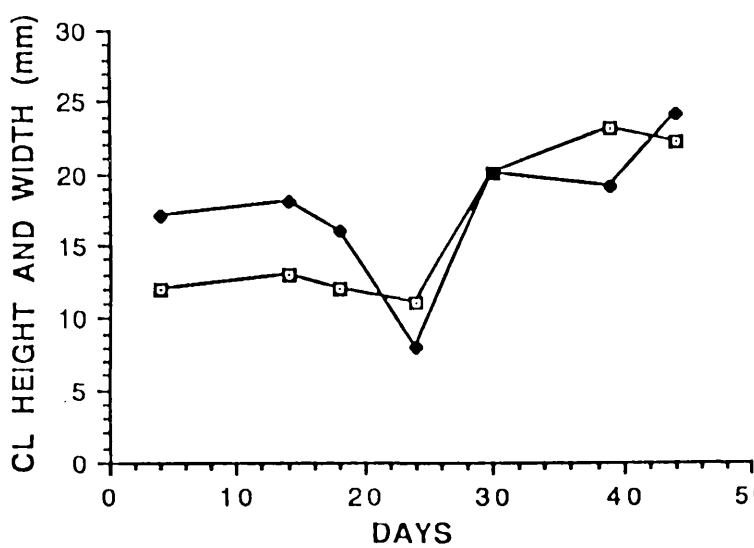

FOLLICULAR HEIGHT (mm)
DAYS
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50

COW 2

(a)


PROG.(ng/ml)
AREA CL (cm 2)
DAYS
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
0
10
20
30
40
50

(b)


CL HEIGHT AND WIDTH (mm)
DAYS
0
5
10
15
20
25
30
0
10
20
30
40
50

(c)

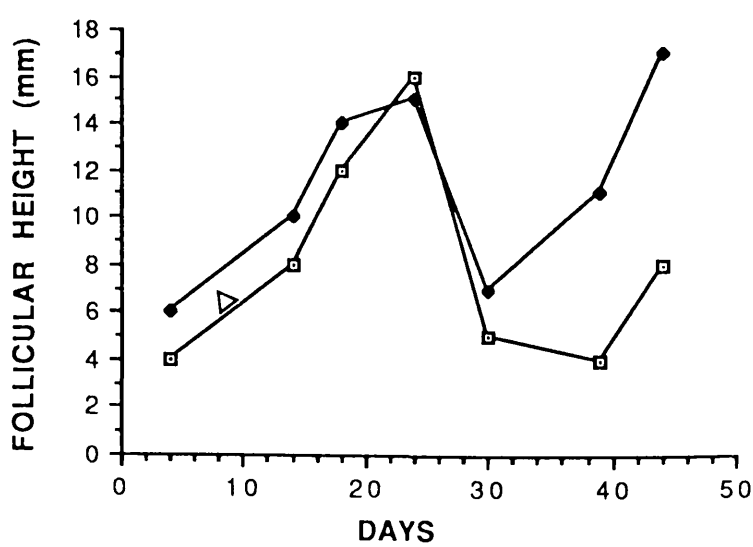
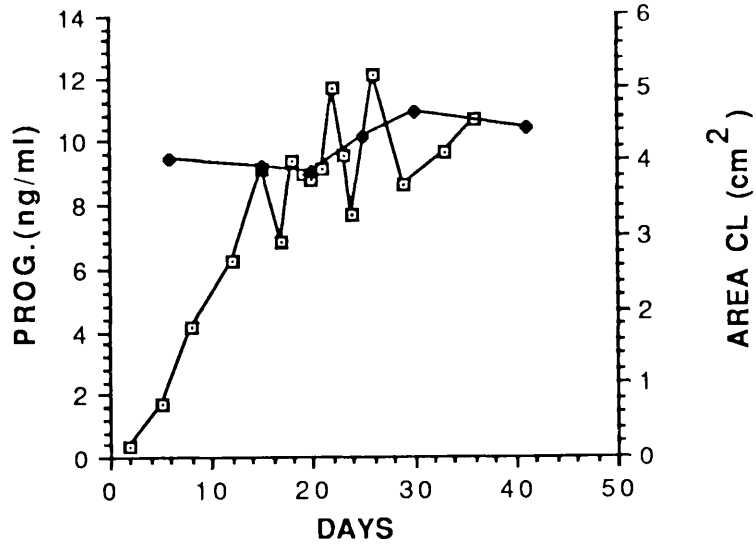

FOLLICULAR HEIGHT (mm)
DAYS
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50

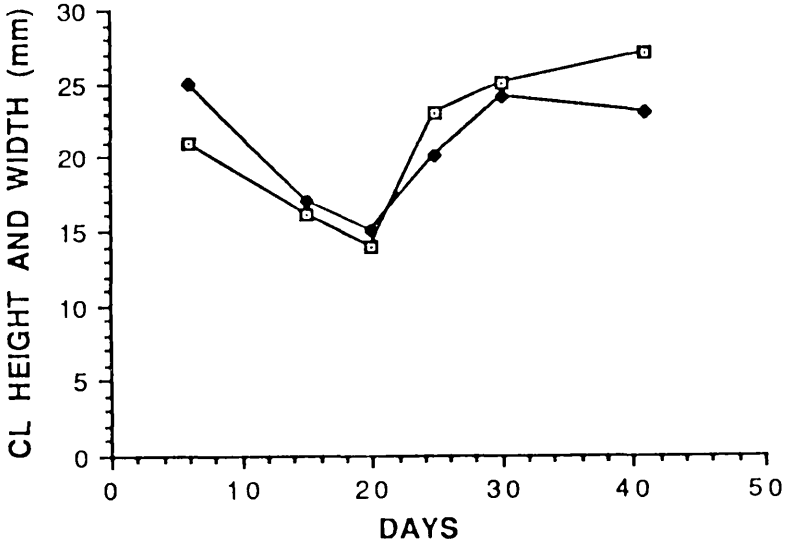
Figure 6. Changes in plasma progesterone concentration (–⊡–) and corpus luteum area (–◆–) **(panel a)**, corpus luteum height (–⊡–) and width (–◆–) **(panel b)** and maximum height on the left (–⊡–) and right (–◆–) ovaries **(panel c)** in cow 72 (Group 2) over a period of approximately 40 days from day of insemination (day 0). (↓) day of oestrus. (Δ) corpus luteum bearing ovary.

COW 72

(a)


PROG.(ng/ml)
AREA CL (cm^2)
DAYS
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
0
10
20
30
40
50

(b)


CL HEIGHT AND WIDTH (mm)
DAYS
0
5
10
15
20
25
30
0
10
20
30
40
50

(c)

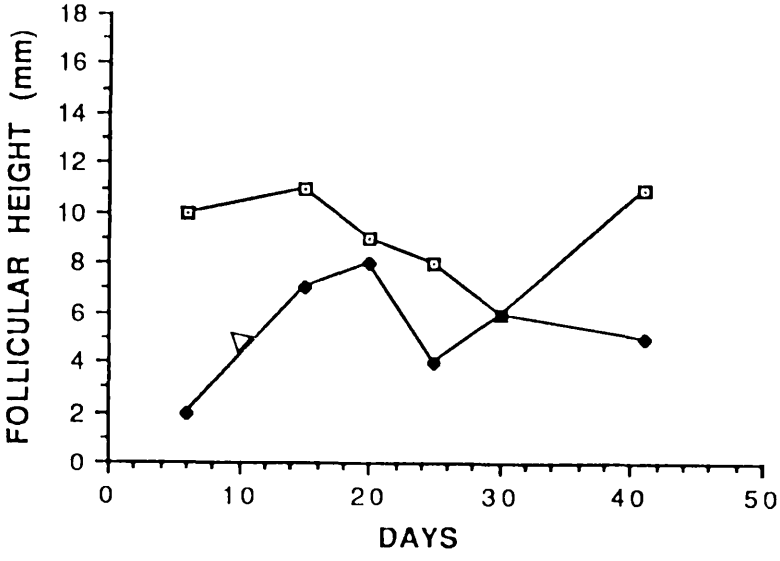
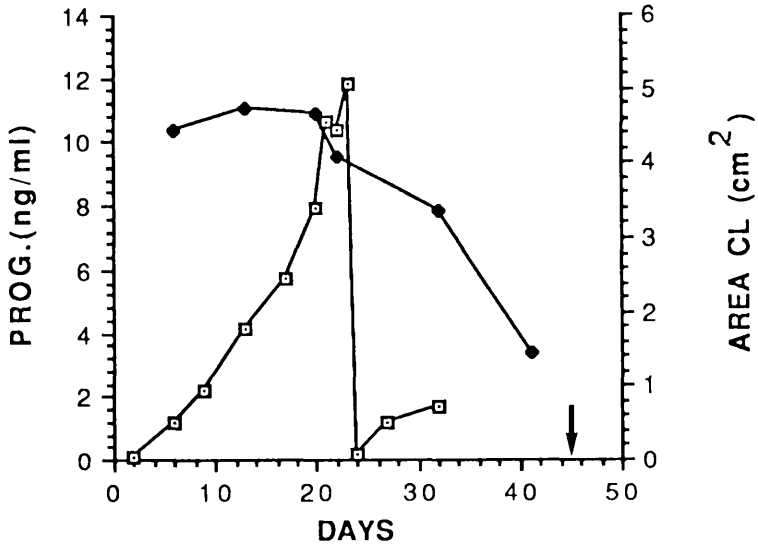

FOLLICULAR HEIGHT (mm)
DAYS
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50

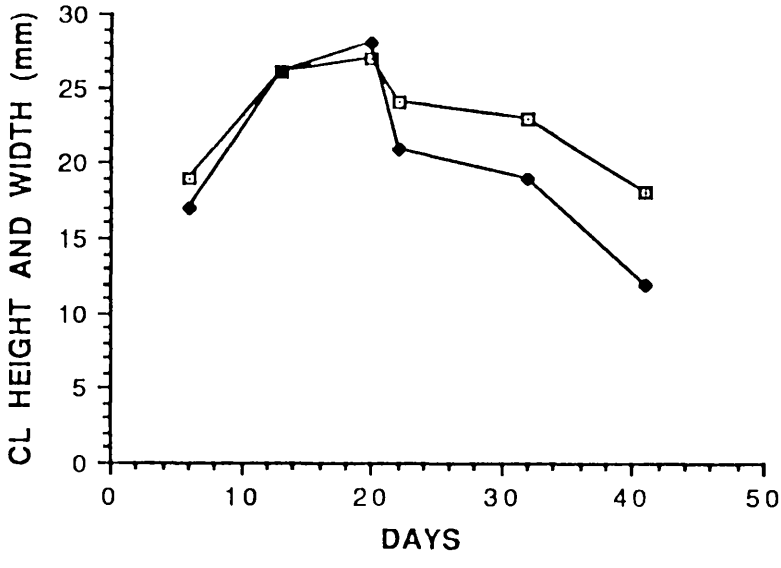
Figure 7. Changes in plasma progesterone concentration (–⊡–) and corpus luteum area (–◆–) **(panel a)**, corpus luteum height (–⊡–) and width (–◆–) **(panel b)** and maximum follicle height on the left (–⊡–) and right (–◆–) ovaries **(panel c)** in cow 72 (Group 2) over a period of approximately 40 days from day of insemination (day 0). (Δ) corpus luteum bearing ovary.

COW 72

(a)


PROG.(ng/ml)
AREA CL (cm^2)
DAYS
0
2
4
6
8
10
12
14
0
1
2
3
4
5
6
0
10
20
30
40
50

(b)


CL HEIGHT AND WIDTH (mm)
DAYS
0
5
10
15
20
25
30
0
10
20
30
40
50

(c)

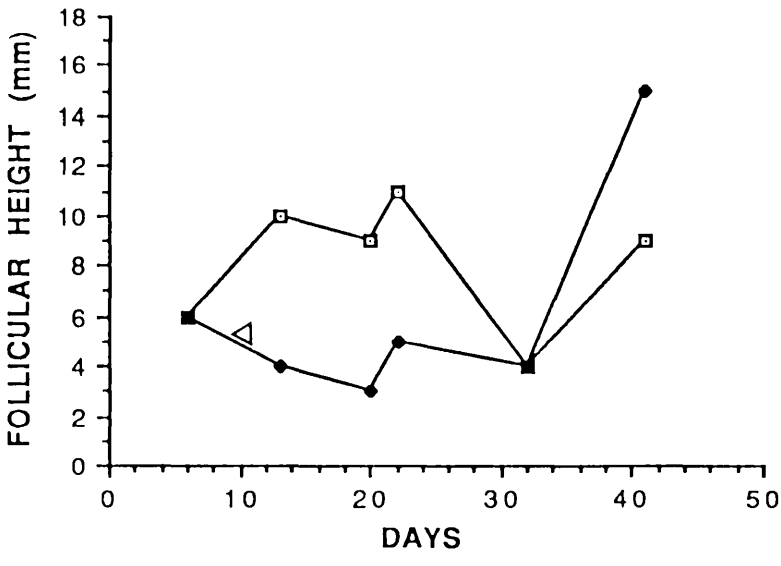
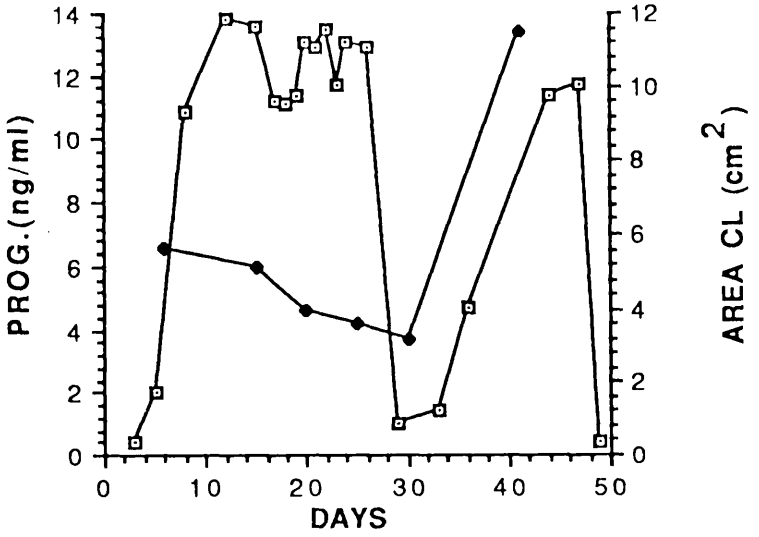

FOLLICULAR HEIGHT (mm)
DAYS
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50

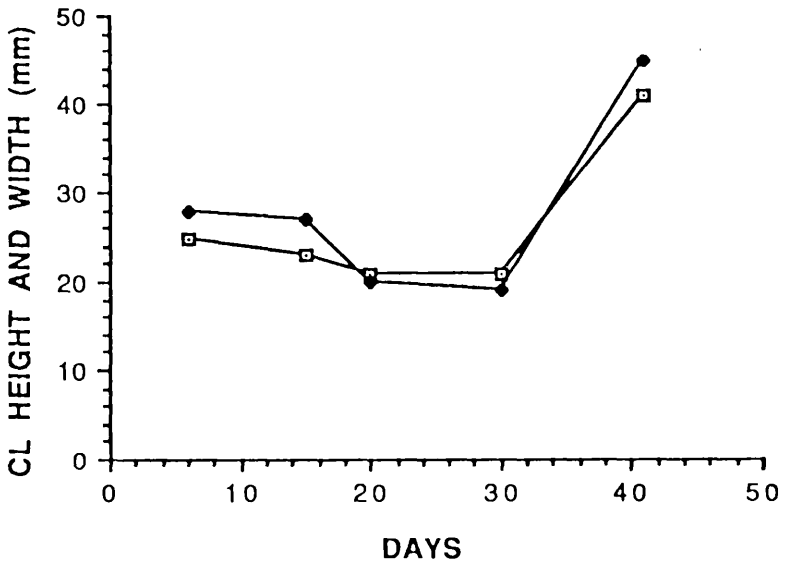
Figure 8. Changes in plasma progesterone concentration (⊣⊡⊢) and corpus luteum area (⊣◆⊢) **(panel a)**, corpus luteum height (⊣⊡⊢) and width (⊣◆⊢) **(panel b)** and maximum follicle height on the left (⊣⊡⊢) and right (⊣◆⊢) ovaries **(panel c)** in cow 1 (Group 2) over a period of approximately 50 days from day of insemination (day 0). (Δ) corpus luteum bearing ovary.

COW 1

(a)


PROG.(ng/ml)
AREA CL (cm^2)
DAYS
0
2
4
6
8
10
12
14
0
10
20
30
40
50

(b)


CL HEIGHT AND WIDTH (mm)
DAYS
0
10
20
30
40
50

(c)

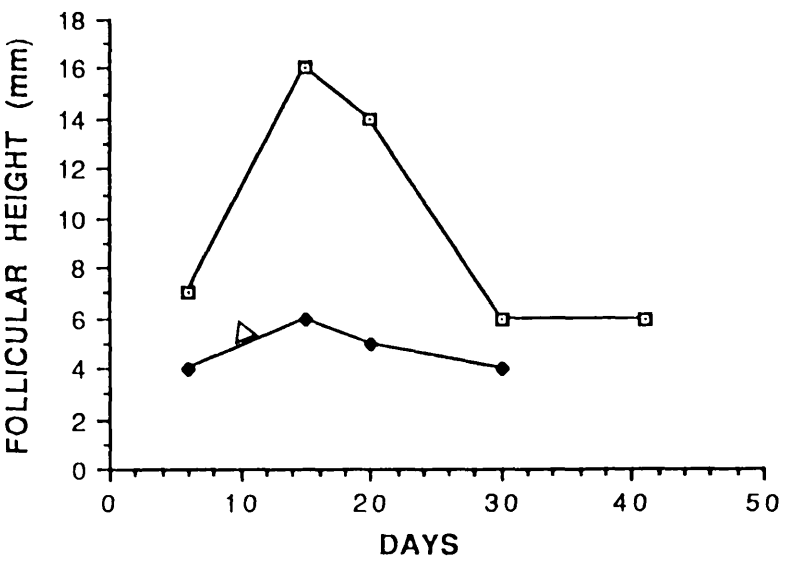

FOLLICULAR HEIGHT (mm)
DAYS
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50

Figure 9. Ultrasound images of (a) an ovary (b) a follicle (c) a corpus luteum with a centrally placed lacuna (d) uterine horn (transverse section). Black arrow heads in figure d indicate myometrium and white arrow heads indicate endometrium (e) an embryonic vesicle within the uterine horn (f) a longitudinal section of cervix.

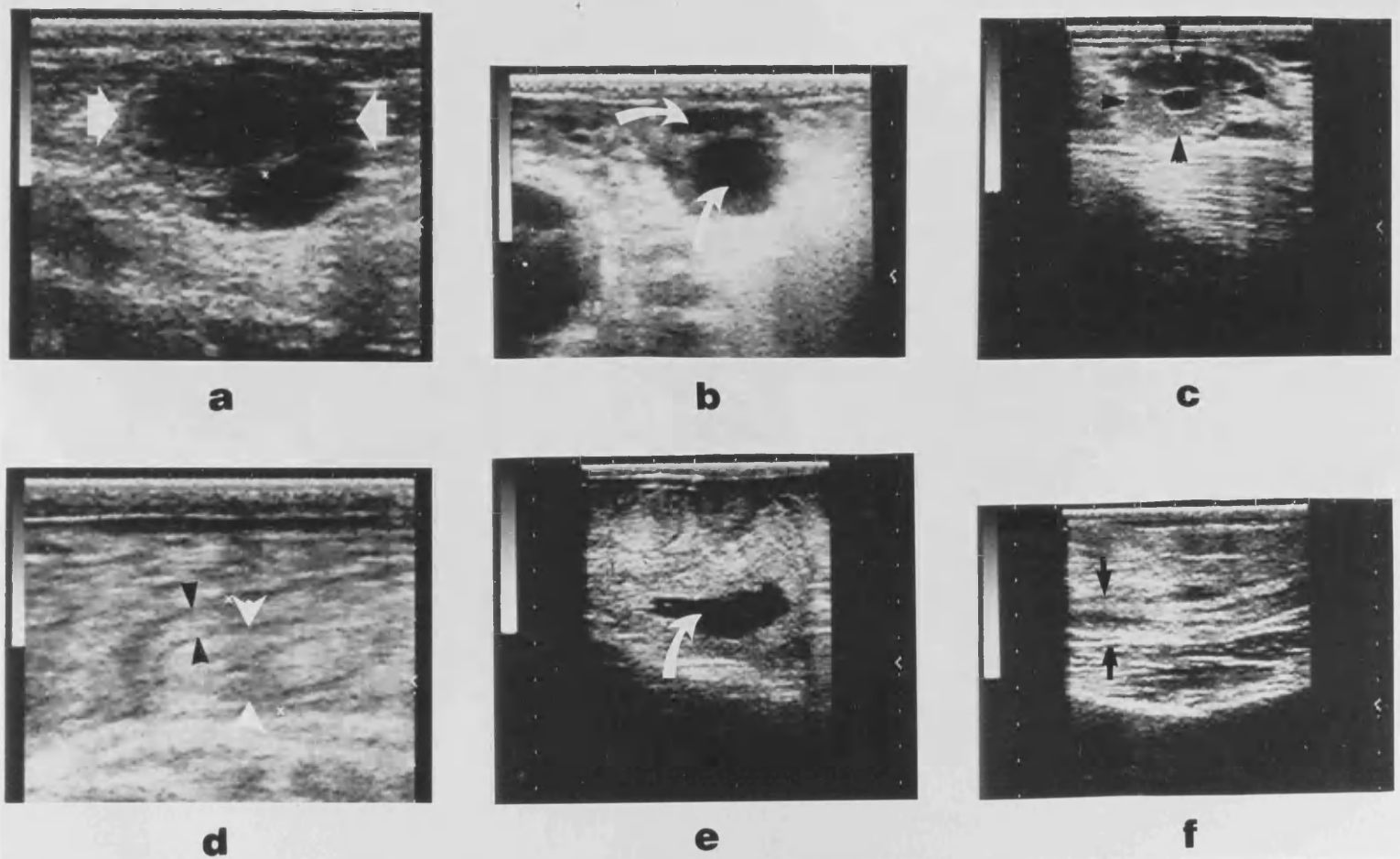

a
b
c
d
e
f

Figure 10. Ultrasound images of the left ovary in cow 34 (Group 1) on (a) day 8 (b) day 15 (c) day 20 (d) day 26 (e) day 30 (f) day 42. Black arrows indicate the corpus luteum.

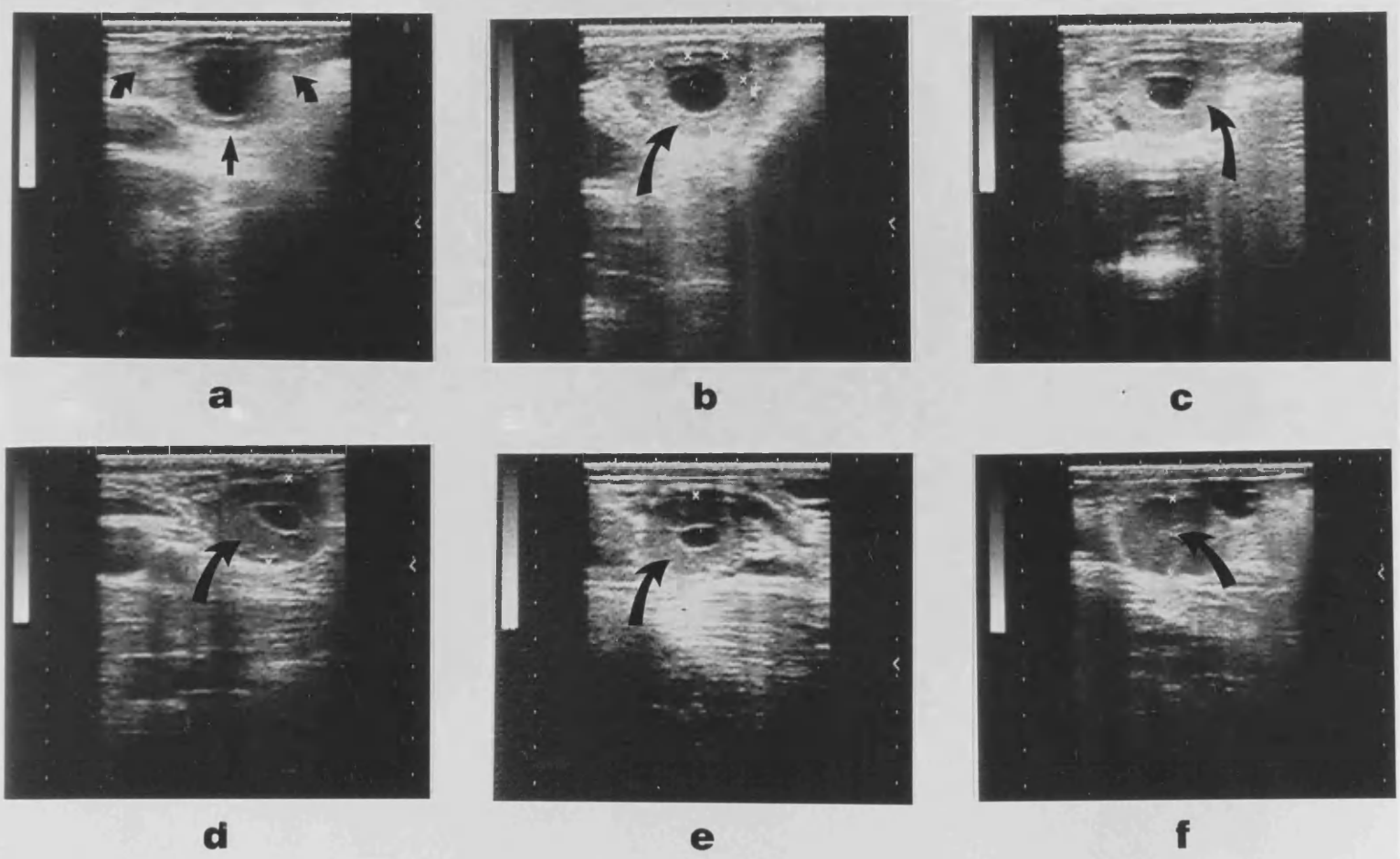

a
b
c
d
e
f

Figure 11. Ultrasound images of the right ovary in cow 34 (Group 1) on (a) day 8 (b) day 15 (c) day 20 (d) day 26 (e) day 30 (f) day 42. Black arrows indicate the corpus luteum.

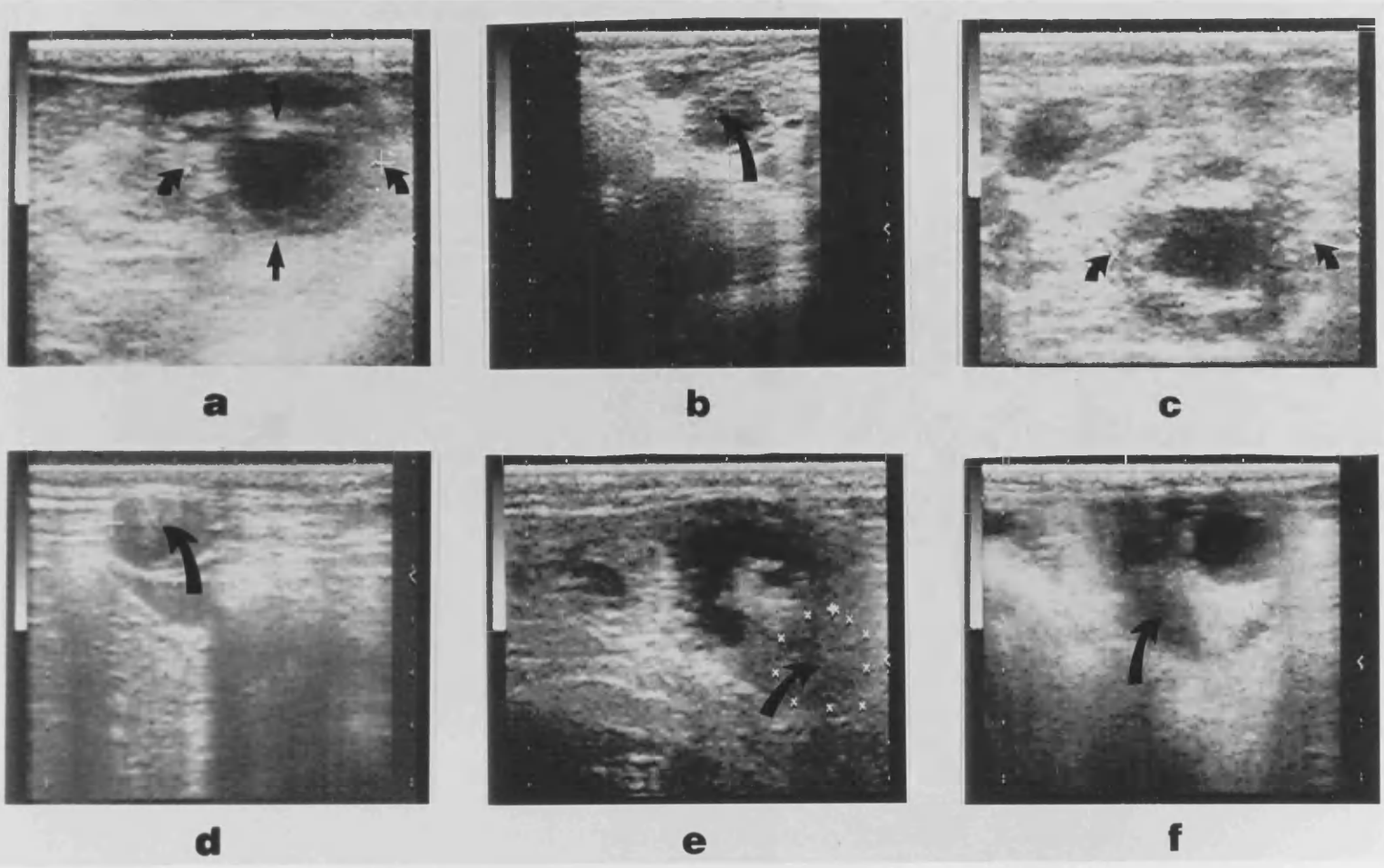

a
b
c
d
e
f

Figure 12. Ultrasound images of the left uterine horn (transverse section) in cow 34 (Group 1) on (a) day 8 (b) day 15 (c) day 20 (d) day 26 (e) day 30 (f) day 42. Black arows indicate the limits of the uterine horn. In Figure b to d white arrows indicate the embryonic vesicle. In Figure e and f white arrows indicate the conceptus.

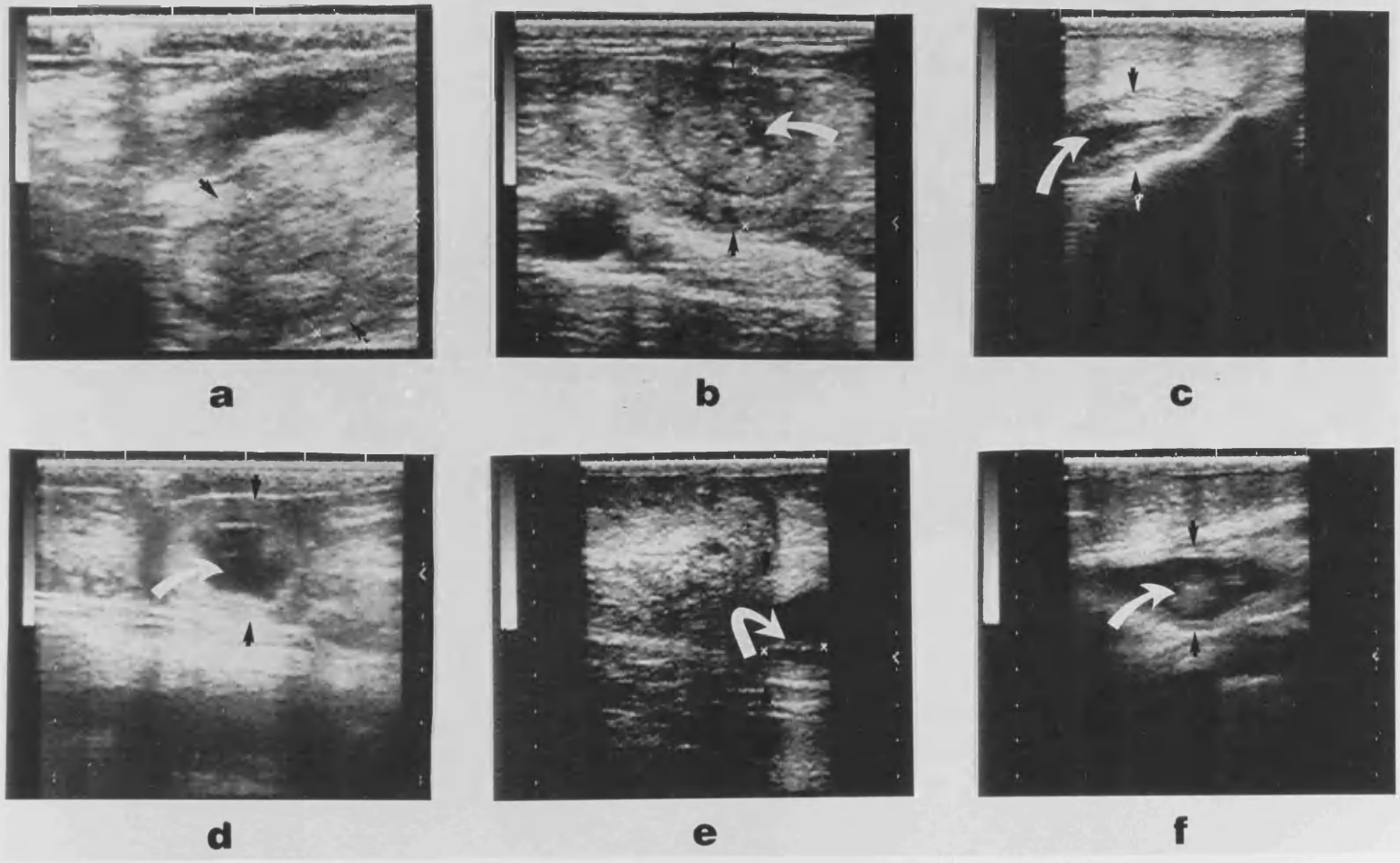

a
b
c
d
e
f

Figure 13. Ultrasound images of the right uterine horn (transverse section) in cow 34 (Group 1) on (a) day 8 (b) day 15 (c) day 20 (d) day 26 (e) day 30 (f) day 42. Black arrows indicate the limits of the uterine horn.

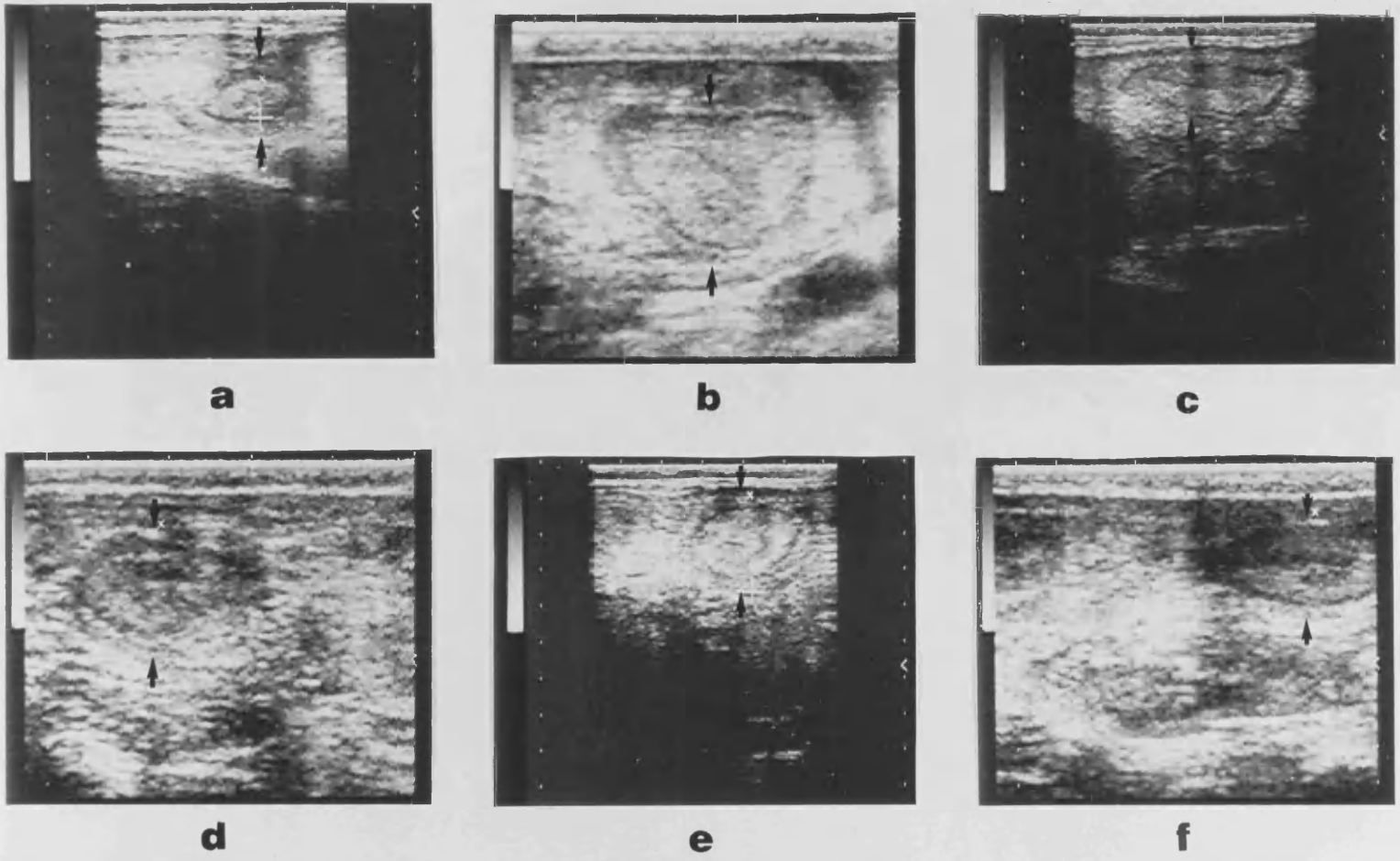

a
b
c
d
e
f

Figure 14. Ultrasound images of the left ovary in cow 29 (Group 2) on (a) day 4 (b) day 14 (c) day 18 (d) day 24 (e) day 30 (f) day 41. In Figures a, c, d, e and f the white arrows indicate the limits of the ovary. In Figure b the white arrow indicates a follicle.

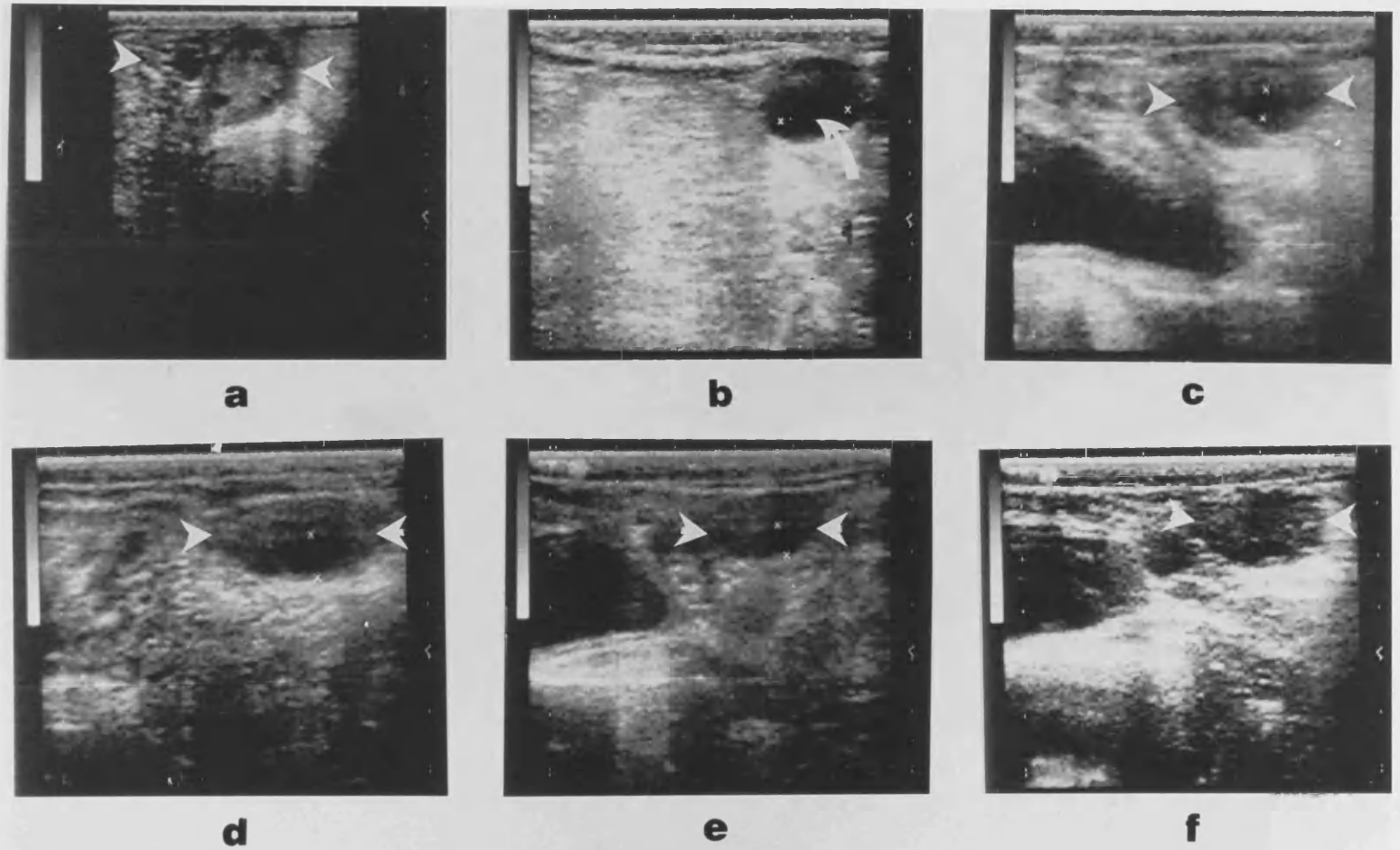

a
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Figure 15. Ultrasound images of the right ovary in cow 29 (Group 2) on (a) day 4 (b) day 14 (c) day 18 (d) day 24 (e) day 30 (f) day 41. Arrows indicate the corpus luteum which has been encircled by crosses in Figure c, e and f for clearer identification.

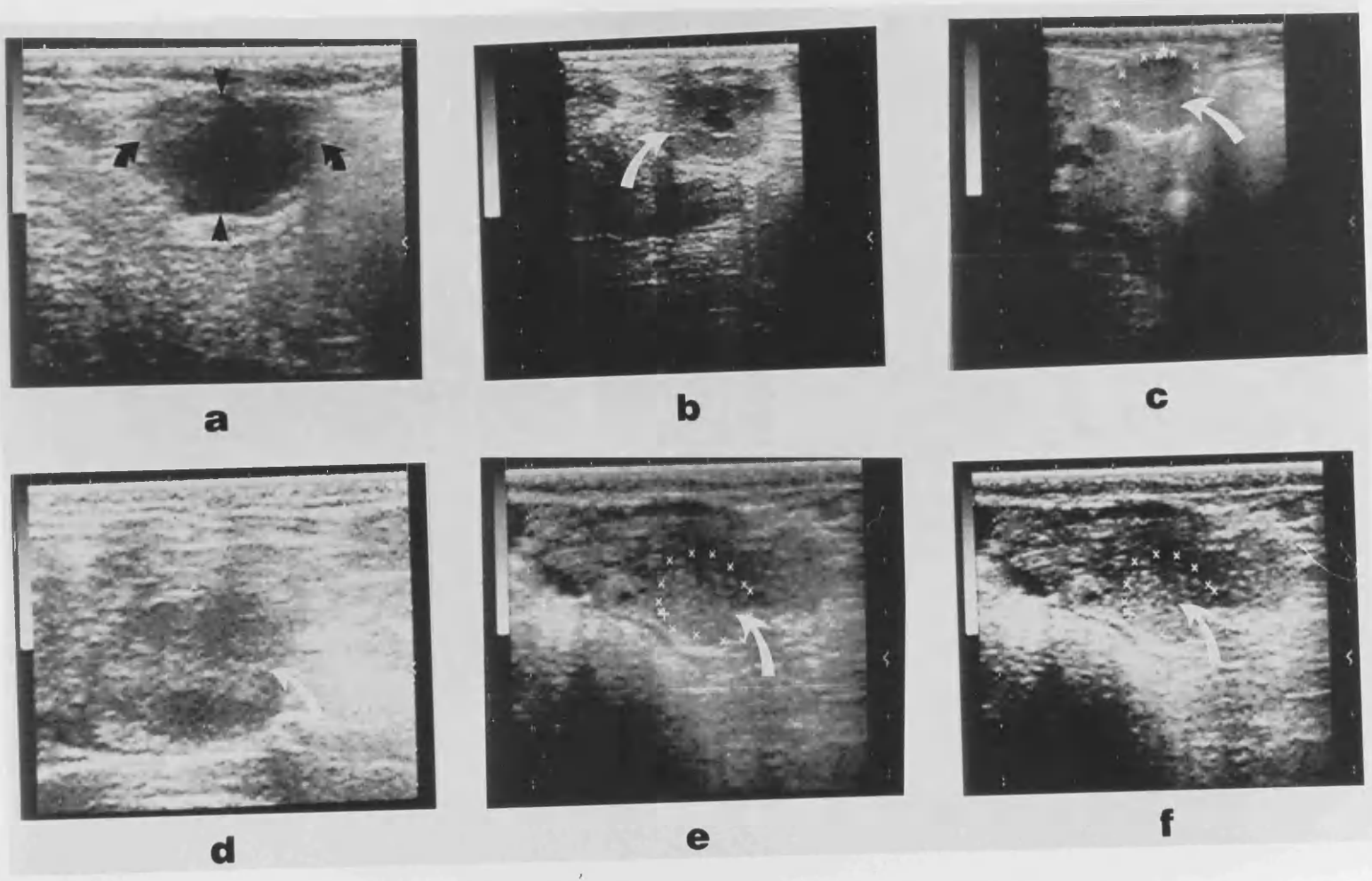
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Figure 16. Ultrasound images of the right uterine horn (transverse section) in cow 29 (Group 2) on (a) day 4 (b) day 14 (c) day 18 (d) day 24 (e) day 30 (f) day 41. Black arrows define the limits of the uterine horn. White arrows in Figure c and d indicate the embryonic vesicle. White arrows in Figure e and f indicate the conceptus.

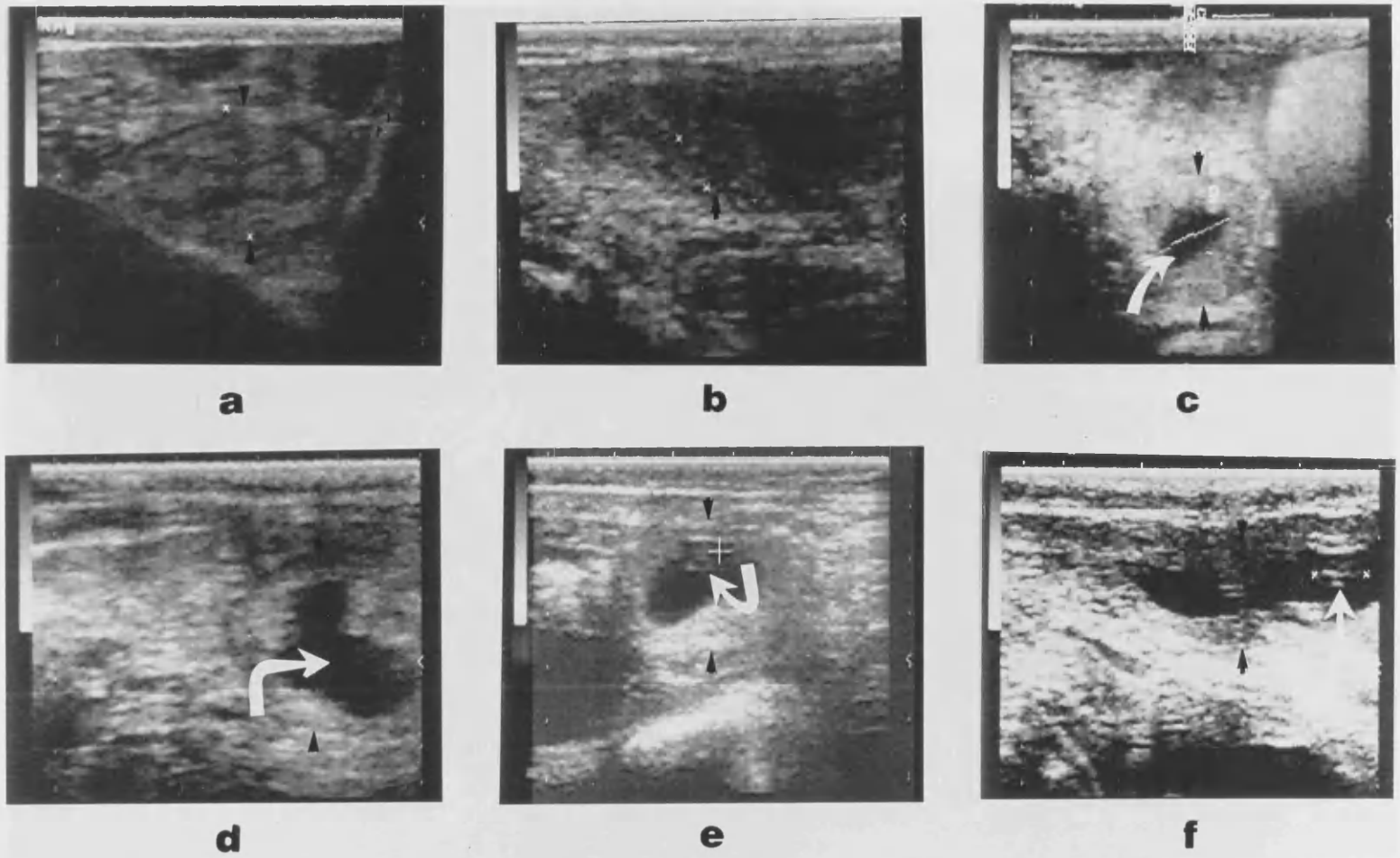
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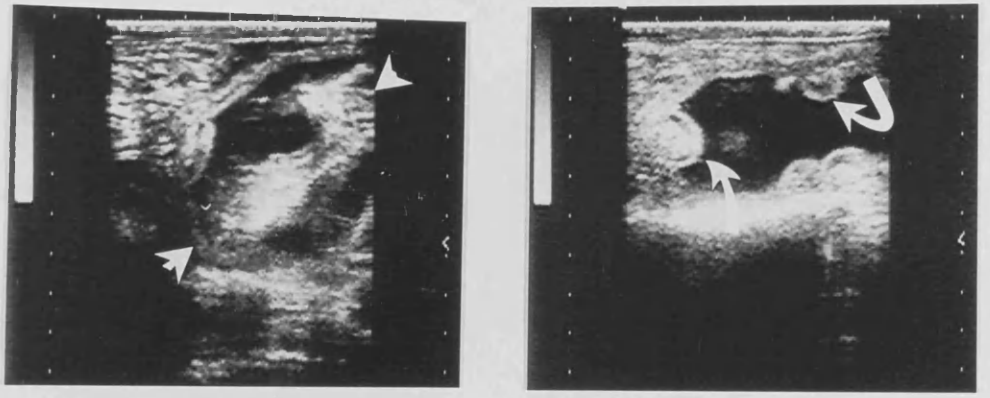
Figure 17. Ultrasound images of the right uterine horn in cow 29 (Group 2) on day 63 showing the fetus in (a) outlined by arrows and cotyledons in (b) arrowed.



a

b

Figure 18. Ultrasound images from cow 10 (Group 2) of the right ovary on (a) day 4 (b) day 16 (c) day 20 and of the left ovary on (d) day 4 (e) day 16 (f) day 20. White arrows and crosses in Figures a to c indicate the corpus luteum. In Figure d, white arrows indicate the limits of the ovary. In Figure e and f, white arrows indicate follicles.

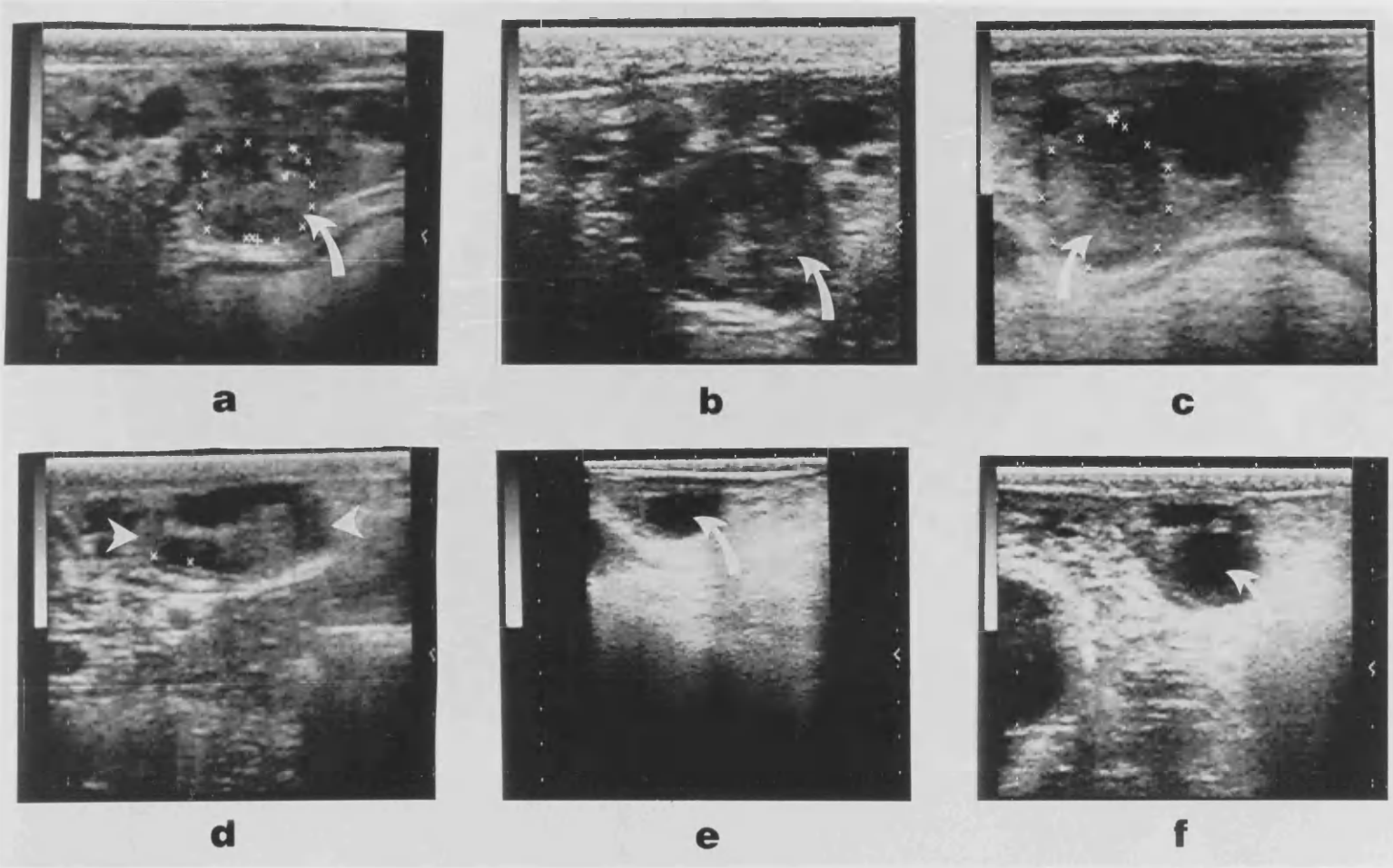

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Figure 19. Ultrasound images from cow 10 (Group 2) of the right uterine horn (transverse section) on (a) day 4 (b) day 16 (c) day 20 and of the left uterine horn (transverse section) on (d) day 4 (e) day 16 (f) day 20. Arrows or crosses indicate the limits of the uterine horn.

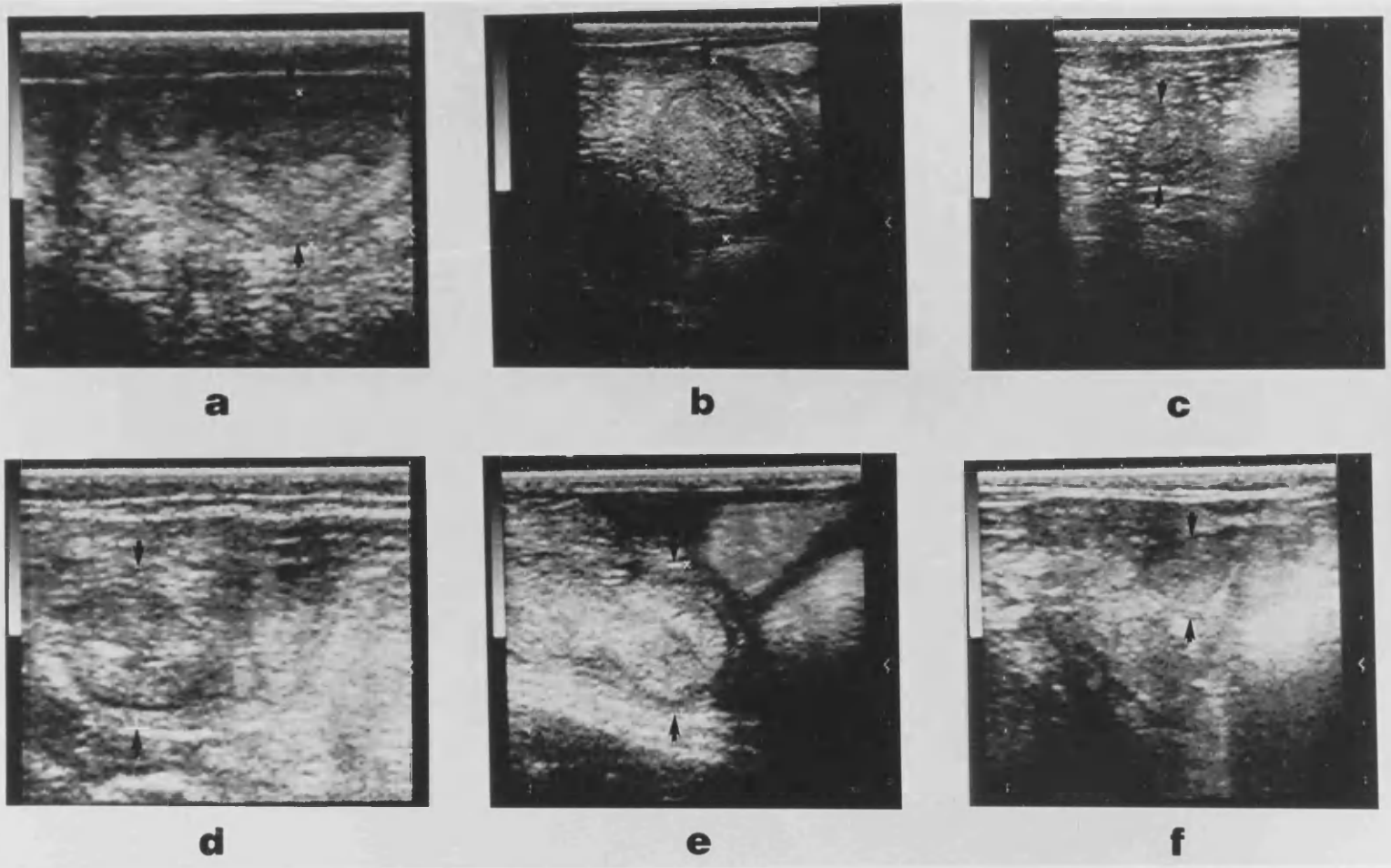
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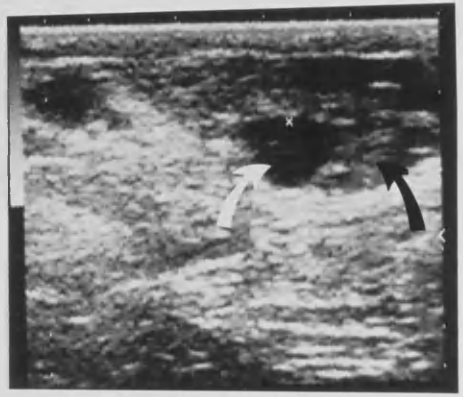
Figure 20. Ultrasound images of the left ovary in cow 2 (Group 2) on (a) day 4 (b) day 14 (c) day 18 (d) day 24 (e) day 30. Black arrows indicate the corpus luteum and white arrows indicate follicles.

a

b

c

d



e

Figure 21. Ultrasound images of the right ovary in cow 2 (Group 2) on (a) day 4 (b) day 14 (c) day 18 (d) day 24 (e) day 30. White arrows in Figure a and b indicate limits of the ovary. White arrows in Figure c and d indicate follicles. Black arrows in Figure e indicate the boundary of the corpus luteum.

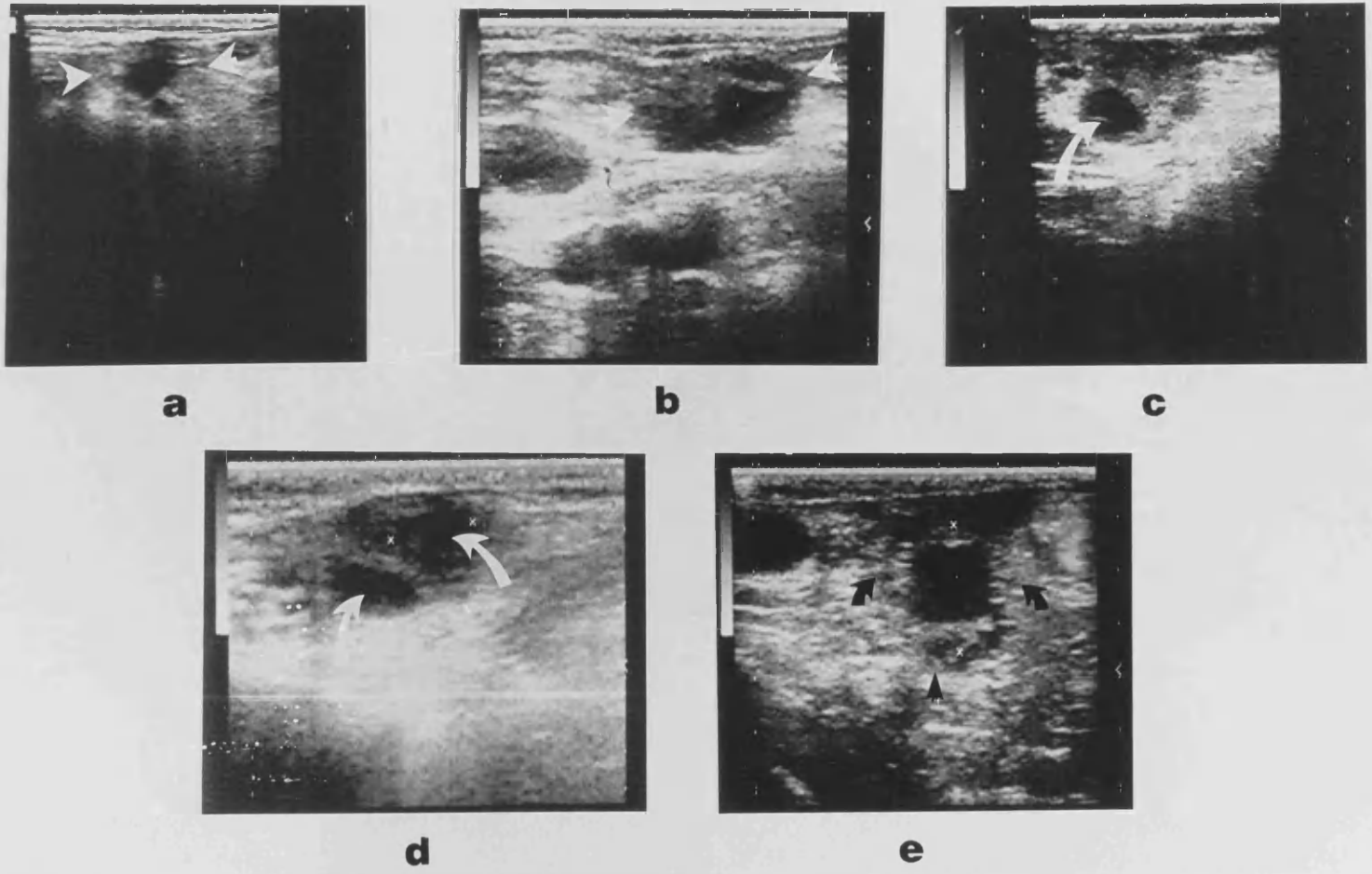

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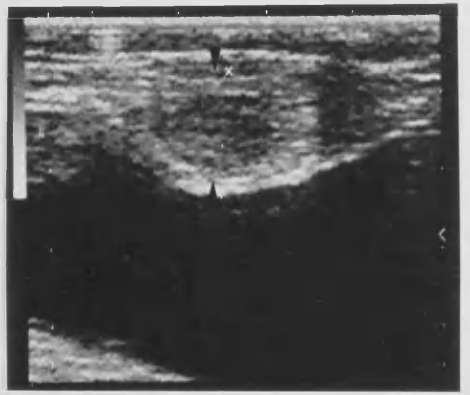
Figure 22. Ultrasound images of the left uterine horn (transverse section) in cow 2 (Group 2) on (a) day 4 (b) day 14 (c) day 18 (d) day 24 (e) day 30. Black arrows indicate limits of the uterine horn.

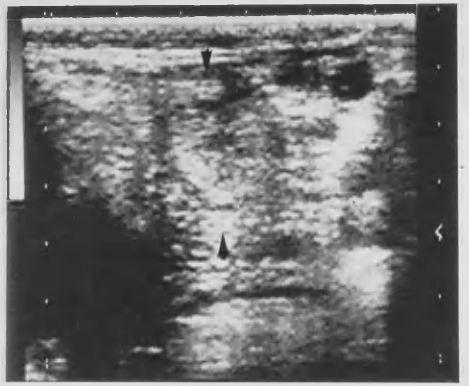
a

b



c

d



e

Figure 23. Ultrasound images of the right uterine horn (transverse section) in cow 2 (group 2) on (a) day 4 (b) day 14 (c) day 18 (d) day 24 (e) day 30. Arrows indicate the limits of the uterine horn.

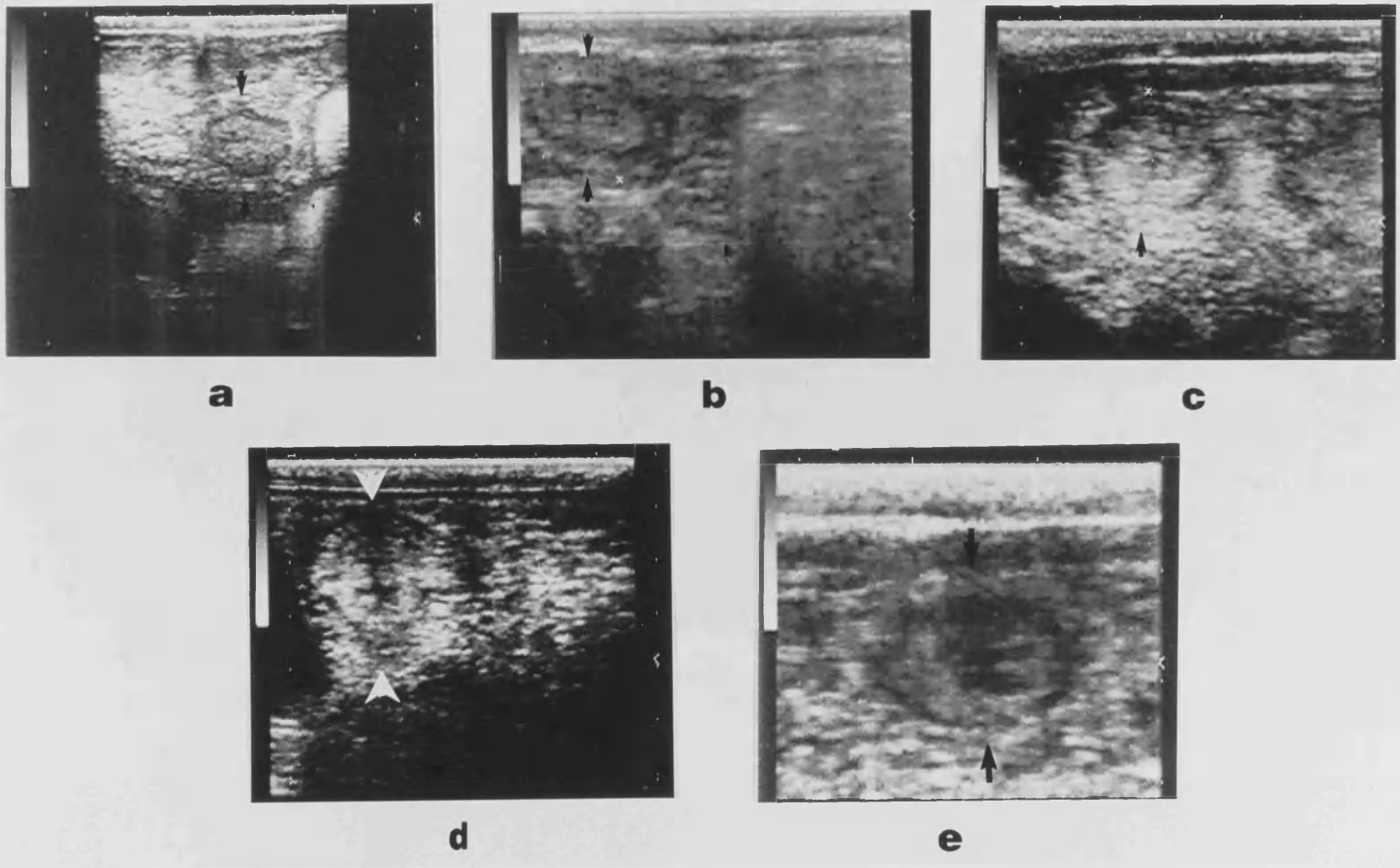

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Figure 24. Ultrasound images of the left ovary in cow 72 (Group 2) on (a) day 6 (b) day 13 (c) day 20 (d) day 22 (e) day 32 (f) day 41. White arrows in Figure a, b, d, e and f indicate follicles. Black arrows in Figure c limits the position of the ovary.

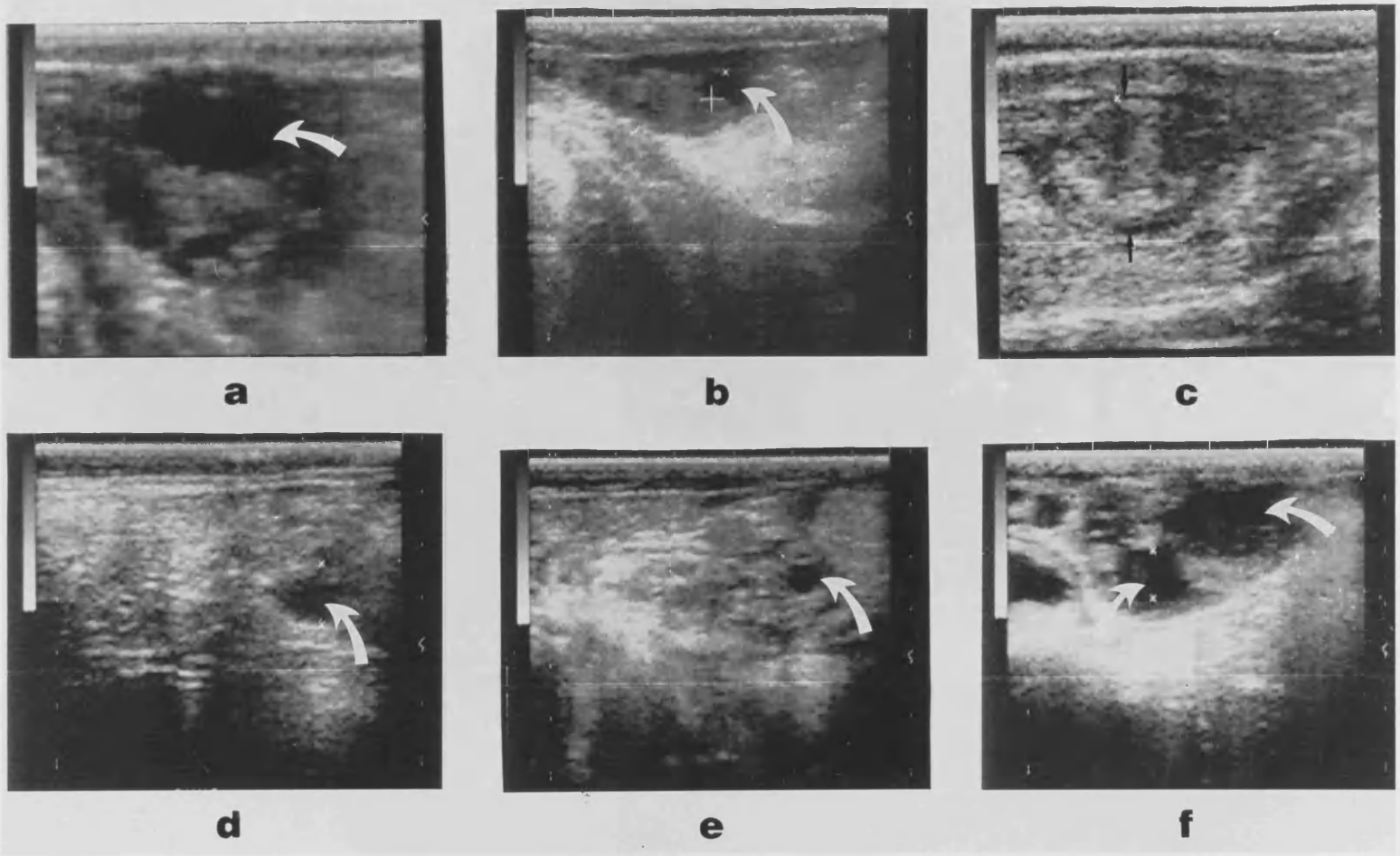
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Figure 25. Ultrasound images of the right ovary in cow 72 (Group 2) on (a) day 6 (b) day 13 (c) day 20 (d) day 22 (e) day 32 (f) day 41. Black arrows indicate the corpus luteum which has been encircled with crosses in Figures b, c and d for clearer identification. The white arrow in Figure f indicates a follicle.

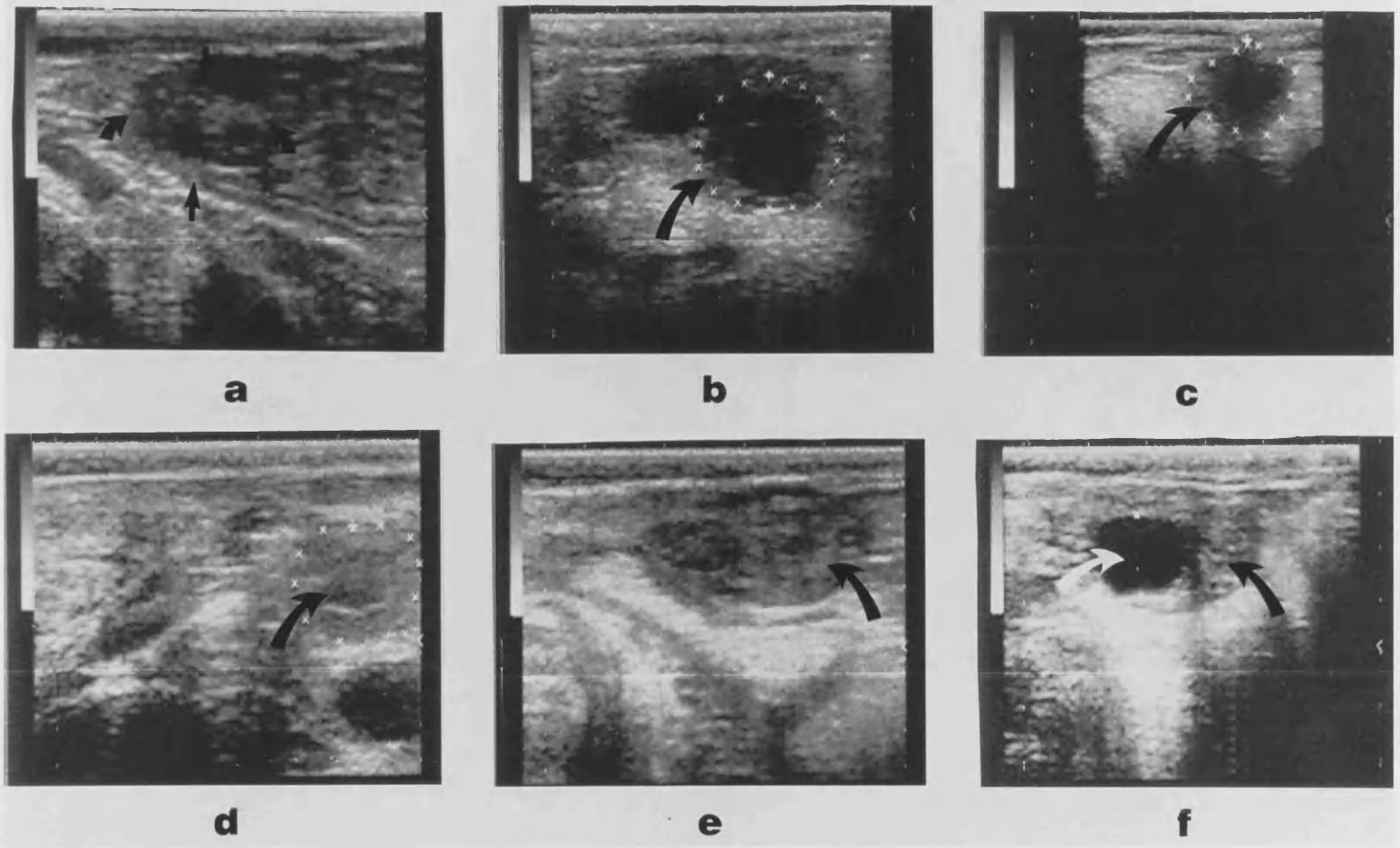
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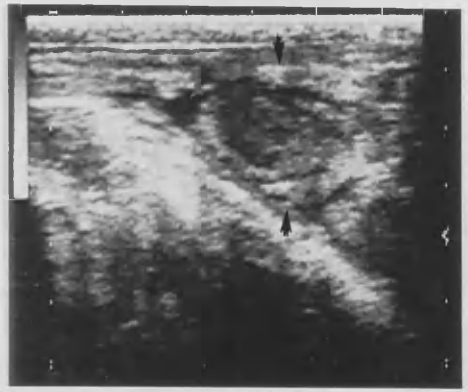
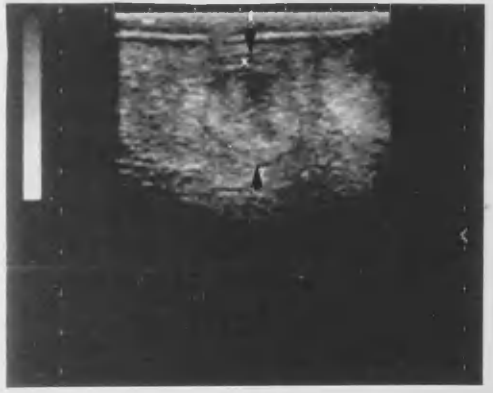
Figure 26. Ultrasound images of the left uterine horn (transverse section) in cow 72 (Group 2) on (a) day 6 (b) day 13 (c) day 20 (d) day 22 (e) day 32 (f) day 41. Black arrows indicate the limits of the uterine horn.

a



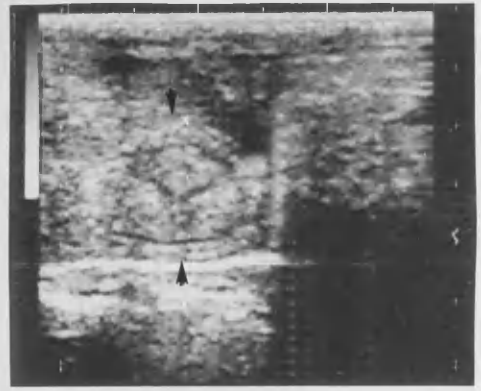
b

c



d

e



f

Figure 27. Ultrasound images of the uterine horn (transverse section) in cow 72 (group 2) on (a) day 6 (b) day 13 (c) day 20 (d) day 22 (e) day 32 (f) day 41. Black arrows in Figure a to f indicate the limits of the uterine horn. White arrow in Figure b indicates the embryonic vesicle. White arrow in Figure c indicates the conceptus. White arrow in figure d indicates the lumen of the uterine horn. White arrow in Figure e indicates snow-like appearance in the lumen of the uterine horn.

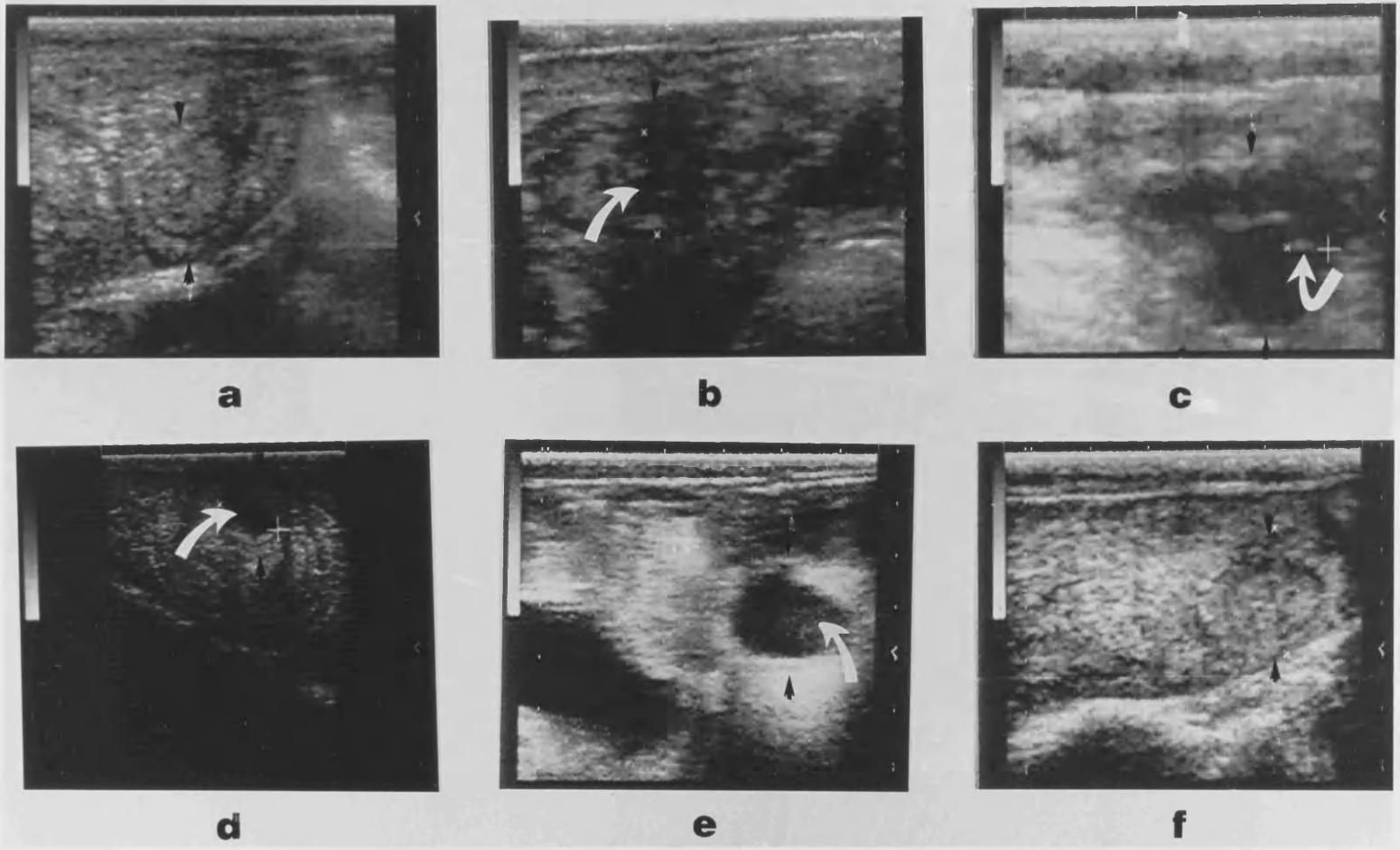

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Figure 28. Ultrasound images of the right ovary in cow 72 (Group 2) on (a) day 6 (b) day 15 (c) day 20 (d) day 25 (e) day 30 (f) day 41. White arrows indicate the corpus luteum.

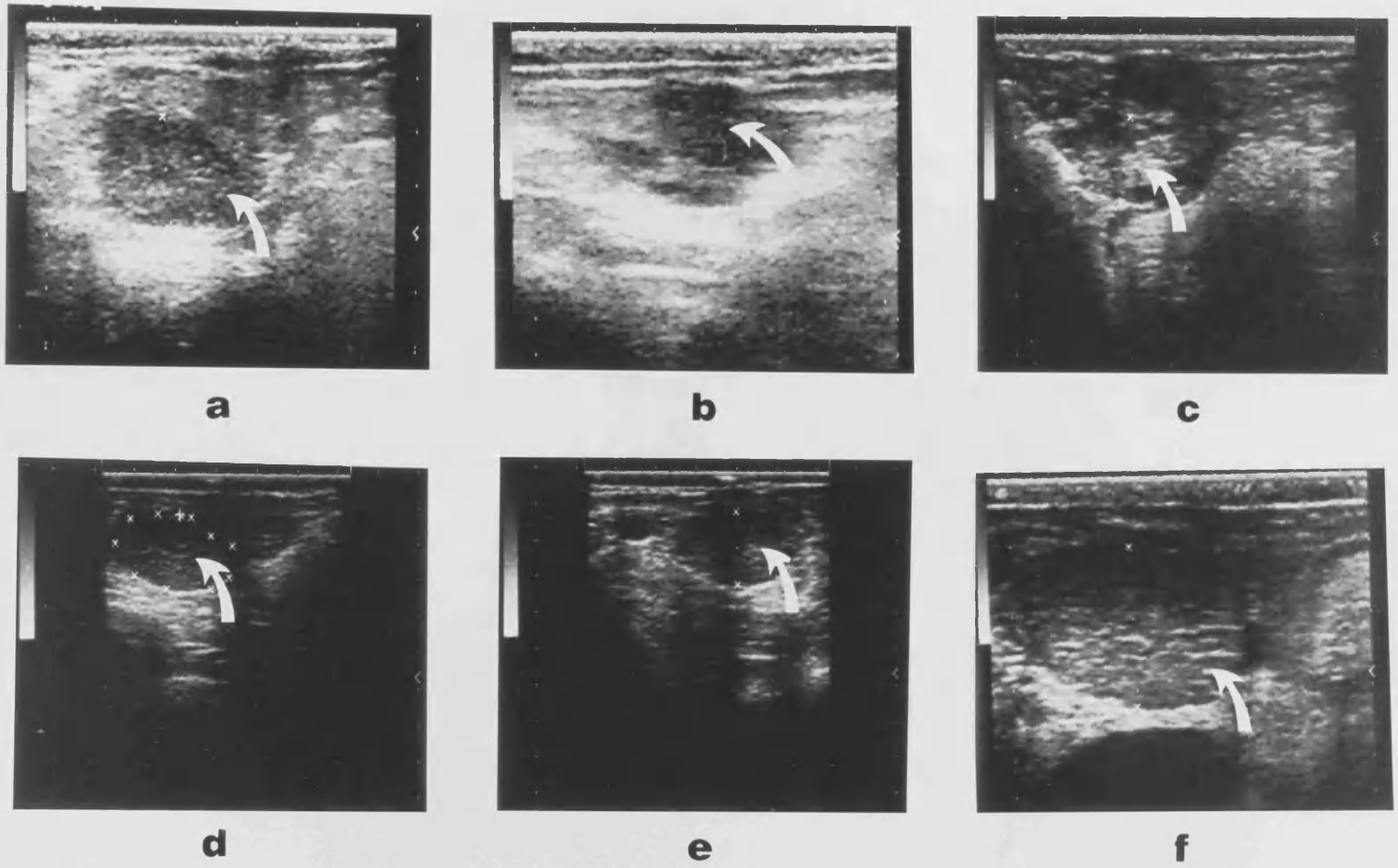
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Figure 29. Ultrasound images of the right uterine horn (transverse section) in cow 72 (Group 2) on (a) day 6 (b) day 15 (c) day 20 (d) day 25 (e) day 30 (f) day 41. White arrows heads in Figure a to f indicate the limits of the uterine horn. White arrow in Figure c indicates the embryonic vesicle. The white arrows in Figures e and f indicate the conceptus.

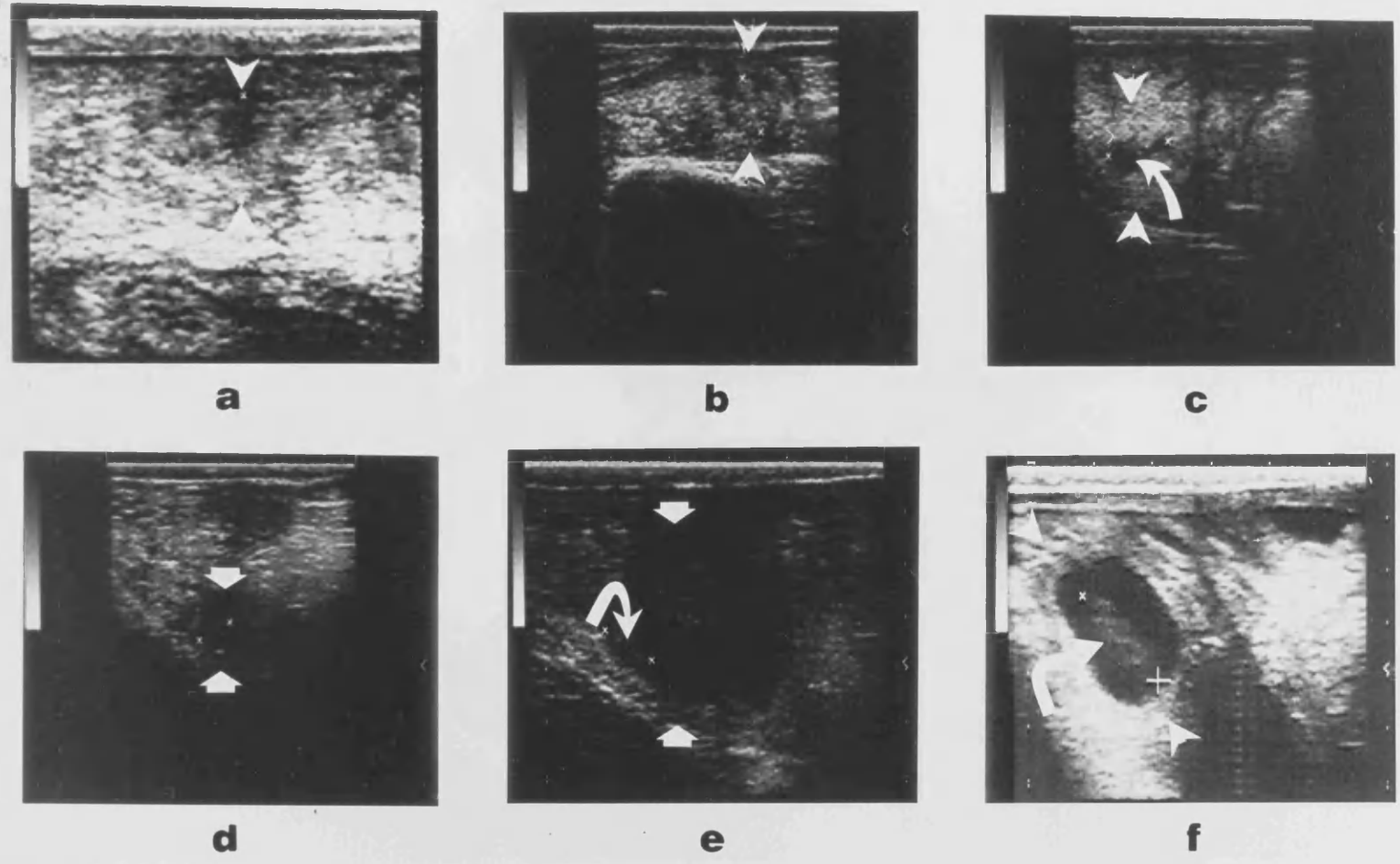
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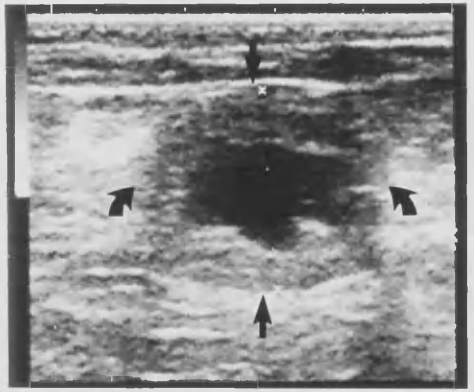
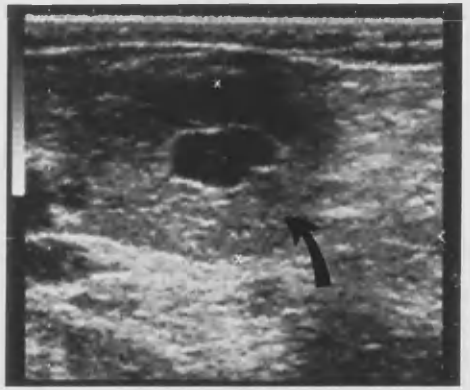
Figure 30. Ultrasound images of the right ovary in cow 1 (Group 2) on (a) day 6 (b) day 15 (c) day 20 (d) day 25 (e) day 30 (f) day 41. Black arrows in Figures a to e indicate the corpus luteum. White arrow in Figure e indicates a follicle. White arrows in Figure f indicate a luteal cyst.



a



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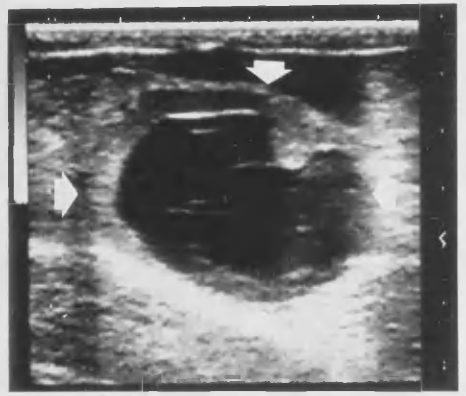

f

Figure 31. Ultrasound images of the left uterine horn (transverse section) in cow 1 (Group 2) on (a) day 6 (b) day 15 (c) day 20 (d) day 25 (e) day 30 (f) day 41. Arrows indicate the limits of the uterine horn.

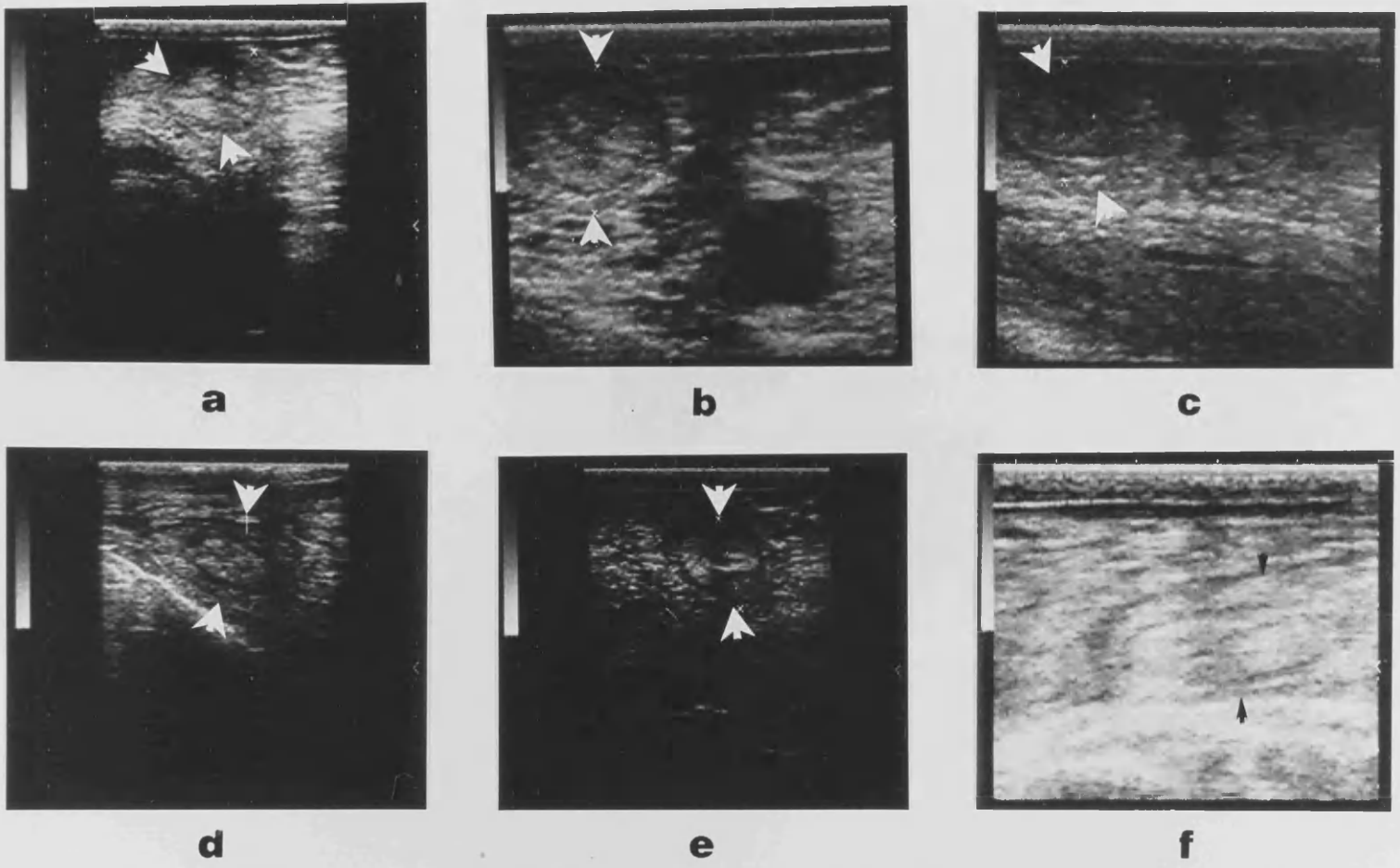
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Figure 32. Ultrasound images of the right uterine horn (transverse section) in cow 1 (Group 2) on (a) day 6 (b) day 15 (c) day 20 (d) day 25 (e) day 30 (f) day 41. Black arrows indicate the limits of the uterine horn. White arrows in Figure c, d and e indicate embryonic vesicle.

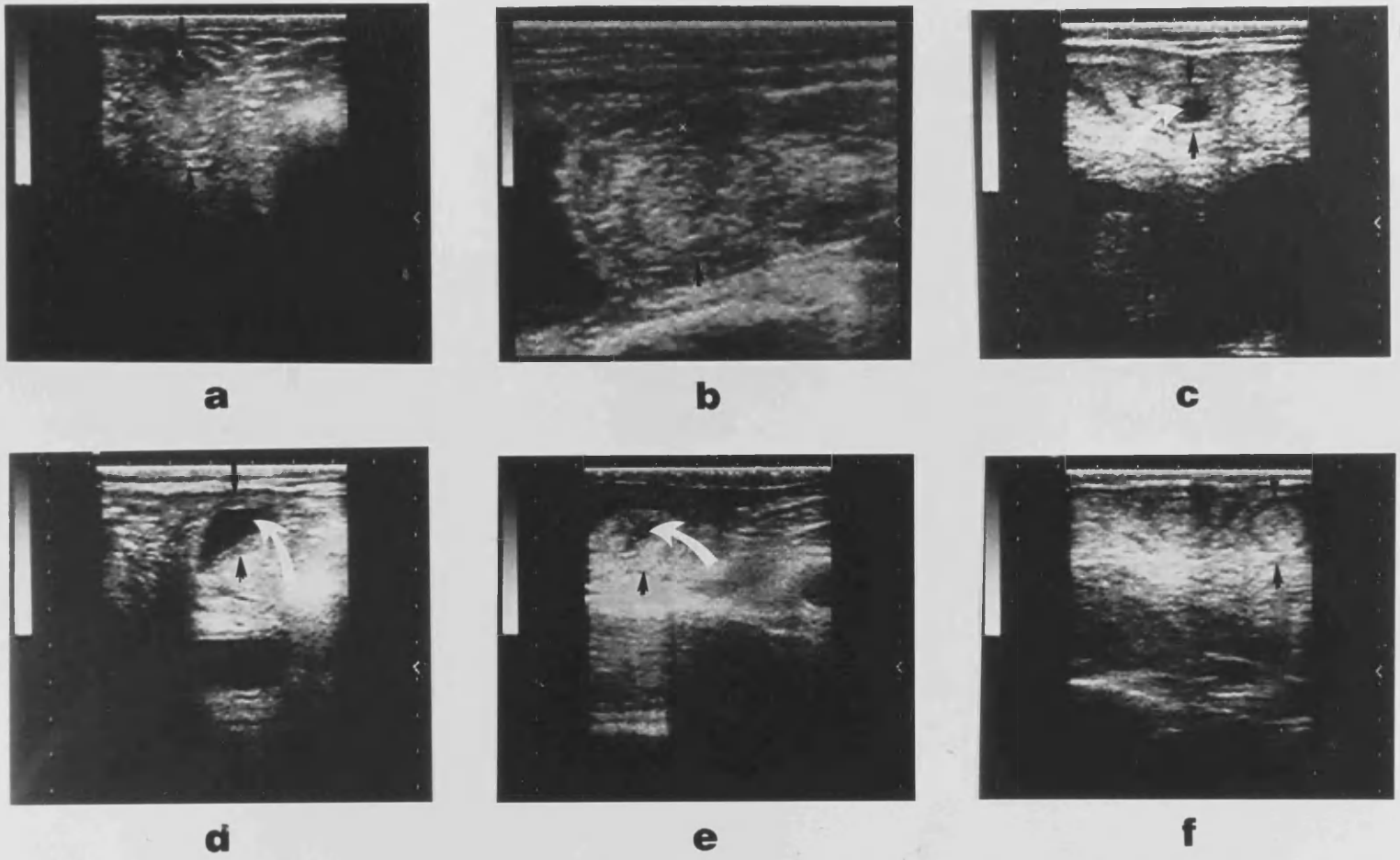
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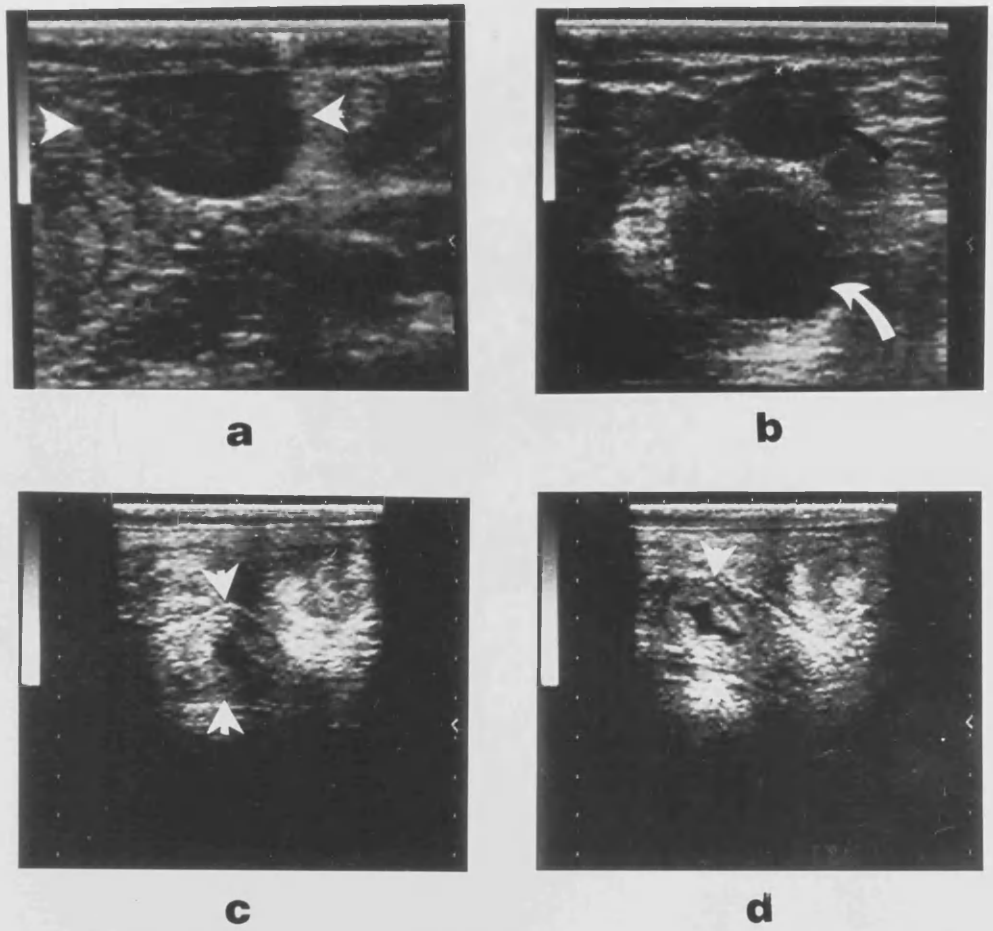
Figure 33. Ultrasound images from cow 1 (Group 2) on day 49 (a) left ovary, white arrows indicate the limits of the ovary; (b) right ovary, white arrow indicates the regressing cystic structure; (c) left horn (transverse section) and (d) right horn (transverse section). White arrows in Figure c and d indicate the limits of the uterine horn.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CHAPTER FOUR

DISCUSSION

Ultrasonography proved to be a useful technique for examining the reproductive tract in both normal and repeat breeder cows. Using ultrasound it was possible to follow the profound morphological changes that took place within the uterus during the oestrous cycle, to detect pregnancy, and to monitor embryonic development and viability. It was effective for monitoring and evaluating ovarian follicles and corpora lutea in normal and repeat breeder cows. In addition, the measurement of plasma progesterone concentration further helped to substantiate and augment ultrasonographic observations.

Images of the ovary were primarily composed of follicles and corpora lutea. Follicles appeared non-echogenic whereas corpora lutea had a different echotexture from the surrounding ovarian tissue which allowed easy distinction between the two. Omran (1989) used a transducer of the same frequency as in this study and was able to describe the formation of corpora haemorrhagica on day 1 and by day 3 was able to distinguish newly formed corpora lutea from the surrounding ovarian stroma. However, Pierson and Ginther (1984a) and Edmondson *et al.*(1986) using a 5MHz transducer failed to differentiate the corpus haemorrhagicum from the rest of the ovarian tissue, though they were able to detect ovulation by the acute disappearance of a large follicle from an ovary. In the present study, the early corpus luteum in both the normal and repeat breeder cows was recognisable since it had a distinct outline and a different echotexture from the surrounding ovarian stroma. By day 15 the corpora lutea had reached maximum size in all cows and had the heterogeneous echotexture which was characteristic of mid-cycle corpora lutea (Omran,1989 & Kastelic *et al.*,1990). All four cows in the control group held to the first service *post partum* and two cows from

the repeat breeder group also conceived in the study (their third service *post partum*) and maintained the corpus luteum. The heterogeneous echotexture of the corpus luteum seen at mid-cycle was maintained in all pregnant animals until the end of the study (Fig. 10, 15 and 28). However, fluctuations in the height, width and area of the corpus luteum were observed in the pregnant cows, especially after day 15 post insemination. Similar fluctuations in height, width and area of the corpus luteum were also observed by Pierson and Ginther (1984a), Omran (1989) and Kastelic *et al.*(1990) and may be due to the varying amounts of luteotrophic proteins (bTP-1) secreted by the developing conceptus at the time of maternal recognition of pregnancy, whose function is to prevent the corpus luteum undergoing luteolysis and to maintain elevated plasma progesterone concentrations. The appearance of a cavity (lacuna) in the centre of the corpus luteum was observed in both the control and the repeat breeder cows (pregnant and non-pregnant) (Fig. 10, 15 and 18). The presence and size of these transient structures did not appear to affect the length of the interovulatory interval, plasma progesterone concentration or establishment of pregnancy. This is compatible with work of Kito *et al.*(1985), Pierson and Ginther (1988) and Kastelic *et al.*(1990). In addition, the results of the present study indicate the potential use of ultrasonography in differentiating a mature corpus luteum from a regressing one (Fig. 10 and 11). A decline in height, width and area and reduction in echogenicity as well as blurring of the outline are all characteristics of a regressing corpus luteum (Pierson and Ginther,1984a & Omran,1989). However, measurement of falling plasma progesterone concentration is also a very reliable indicator of corpus luteum regression. There were a number of occasions in the results where rapid estimation of plasma progesterone concentration would have revealed the onset of luteolysis before any detrimental changes in luteal structure were observed by ultrasonography eg. Cow 72, day 24 (Fig. 6a) and there

were also occasions when quite dramatic decreases in luteal size were not accompanied by lowered progesterone levels and pregnancy was maintained eg. Cow 29, days 24-38 (Fig. 2a). Cow 34 had twin ovulations and this was substantiated by the ultrasonographic detection of two corpora lutea, one on each ovary (Fig. 10 and 11). The one on the left ovary (ipsilateral to the pregnant horn) was maintained throughout the period of observation whereas the one on the right ovary regressed by day 24 and follicles of varying dimensions appeared on the ovary. Work by Mapletoft *et al.*(1976) demonstrated that the gravid horn exerted an antiluteolytic effect on the adjacent ovary and that the effect was exerted through a local utero-ovarian veno-arterial pathway. Similarly, work by McCracken *et al.*(1973) had indicated that a luteolytic factor was transported from the uterine vein to the ovarian artery locally by a countercurrent mechanism. Thus the maintenance of the corpus luteum on the left ovary and the regression of the corpus luteum on the right ovary is further evidence for the local pathway for both maintenance of the corpus luteum in pregnancy and the demise of corpus luteum during luteolysis.

The graphs for plasma progesterone concentration in pregnant cows indicated that plasma progesterone concentration declined between day 16-20 post insemination and then increased again (Fig. 1a, 2a and 7a). During the same period, a slight reduction in the area of the corpus luteum in the pregnant cows (cows 21, 34 and 73 Fig. 1a and cow 72, Fig. 7a) was also evident before it too increased again. Omran (1989) and Kastelic *et al.*(1990) showed that with daily scanning these changes occurred about day 15-17 post insemination in pregnant cows. It is interesting to note that Lukaszewska and Hansel (1980) found no difference in plasma PGF2a concentration in pregnant and non-pregnant cows at day 18. Thus it seems possible that there is early production of PGF2a, which causes some alteration in the morphology of the

luteal cells and in the steroidogenic capacity of the corpus luteum before being counteracted by bTP-1 produced by the developing conceptus. In those cows that returned to service, the decline in plasma progesterone concentration was more rapid and occurred earlier than the regression of corpus luteum, as observed by reduction in height, width, area and echogenicity. Omran (1989) and Kastelic *et al.*(1990) using daily scanning found that plasma progesterone concentration started declining 2-3 days prior to the detectable changes in the echotexture of the corpus luteum. The initial decline in plasma progesterone concentration presumably occurred under the influence of the first peaks of PGF2a released from the uterus (Lukaszewka and Hansel,1980) and this probably affected the binding of LH to the luteal cells and initiated the degenerative changes as described by Knickerbocker *et al.*(1988). Erb *et al.*(1971) found that the plasma progesterone concentration started to decline much earlier than the reduction in weight of excised corpora lutea. Although the approach used by Erb *et al.*(1971) was different from that of the present study, the results are comparable and this further confirms the ability of ultrasonography to detect *in vivo* changes. In the present study, plasma progesterone concentration was at a peak when the corpus luteum reached maximum size and exhibited a heterogeneous echotexture. A high correlation between plasma progesterone concentration and echotexture of the corpus luteum was also reported by Kastelic *et al.*(1990). In contrast, Ott *et al.*(1986) reported only 65% accuracy in correlating luteal status by rectal palpation and plasma progesterone concentration. Watson and Munro (1980) fared even worse and could find no correlation between the size of the corpus luteum as determined by rectal palpation and milk progesterone concentration. Sprecher *et al.*(1989) compared rectal palpation with transrectal ultrasonography for estimating luteal function, as measured by milk progesterone concentration, and found that rectal palpation was 70% accurate whereas ultrasonography was 90% accurate. Thus the results of the

present study as well as those of by Omran (1989), Sprecher *et al.*(1989) and Kastelic *et al.*(1990), indicate that transrectal ultrasonography is more superior than rectal palpation for assessment of corpus luteum function. There was apparently no difference in the echotexture of the corpus luteum between the pregnant cows in the control group and the cows that held to service in the repeat breeder group (Fig. 10, 15 and 28).

Ultrasonography has been used previously to monitor follicular dynamics in cattle (Pierson and Ginther,1987,1988). The present study was not undertaken to test any of the biological hypotheses put forward by a number of researchers concerning follicular dynamics. The main purpose of monitoring follicular dynamics in this study was to assess any difference in pattern of follicular growth between normal and repeat breeder cows. More follicular activity was evident in non-pregnant cows than in pregnant cows (Rexroad and Casida,1975 & Pierson and Ginther,1988). With the exception of cow 73 (Fig. 1c), the diameter of the largest follicle hardly exceeded 10mm in pregnant cows. This essentially agrees with the results of Pierson and Ginther (1988) who found the average diameter of the largest follicle in pregnant cows to be 12mm. The slight variation in height of the largest follicle between the two studies could be attributable to the different frequencies of the transducers used in both studies. In the pregnant cows, follicles on the ovary contralateral to the corpus luteum bearing ovary attained greater dimensions compared to follicles on the corpus luteum bearing ovary (cow 21 and 73, Figure 1c). These results are consistent with the slaughter house study of Rexroad and Casida (1975) and ultrasonographic study of pregnant cows by Pierson and Ginther (1987). The presence of an active corpus luteum during pregnancy may therefore be inhibiting or limiting follicular growth on the ipsilateral ovary by a mechanism which is still unclear. The present results indicate that the ovary is not quiescent during pregnancy and that

follicular activity does continue even though the follicles do not attain the same dimensions as the dominant follicles (Fig. 1c and 2b). On the other hand there was no apparent difference in the pattern of follicular growth between the cows that conceived in the repeat breeder group and the cows in the normal group (Figure 1c, 2c and 7c).

The ultrasonographic appearance of the uterus was influenced by the stage of the oestrous cycle and by pregnancy. In non-pregnant animals striking differences in the appearance of the uterus were seen when comparing oestrus and dioestrus. As oestrus drew near, profound changes in the characteristics visible by ultrasonography were observed such as thickening of the uterine body (evidence of oedema) and accumulation of intrauterine fluid (Fig. 19). Such observations correspond to those described by Fissore *et al.*(1986), Pierson and Ginther (1988) and Omran (1989), who all noted by scanning daily that these characteristics developed 4-5 days prior to oestrus. Development of a heterogeneous echotexture of the endometrium is presumably due to the high plasma oestrogen concentration during the periovulatory period which causes increased tone of the uterus and oedema of the endometrial folds. During dioestrus no fluid was present in the lumen of the uterus nor were any endometrial folds discernible (Fig. 19b, 19e, 22b and 23b). However, the endometrium and myometrium were distinguishable. Similar observations were described by Fissore *et al.*(1986), Pierson and Ginther (1988) and Omran (1989). The fairly homogeneous appearance of the images of the uterine horn were characteristic of dioestrus and were observed during the period associated with maximal progesterone concentration as was shown by Fissore *et al.*(1986).

Pregnancy was confirmed in this study by demonstrating the presence of a discrete non-echogenic circumscribed area in the uterine horn (Fig. 27b). Such structures were identified as embryonic vesicles, and were first detected about day

13 post insemination. The embryo proper could be identified by day 20 as an echogenic structure within the non-echogenic vesicle (Fig. 27c). Omran (1989) used the same frequency transducer for scanning daily and was able to detect these vesicles as early as day 9 and by day 11 the embryo proper was observed. In addition, by day 19 he observed embryonic heartbeat emanating from the echogenic structure as a pulsatile movement. On the other hand, Pierson and Ginther (1984b) used a 5MHz transducer and could not identify the embryonic vesicle until day 12 and the embryo proper and heartbeat until about day 26. Kastelic *et al.*(1989) using a 5MHz transducer were able to confirm pregnancy in 100% cases by day 22 whereas Omran (1989) using 7.5MHz transducer was able to confirm pregnancy in 100% cases by day 17. The present results confirm the potential advantages of using a high frequency transducer for more precise detection of early pregnancy in cattle before the commencement of the next oestrous cycle. This has advantages in terms of being prepared for oestrus detection and repeat insemination. Franco *et al.*(1987) reported that 7.5% of early embryonic death in cattle before day 35 was due to rupture of the amniotic sac during rectal palpation. In contrast there is no report in the literature to date which has demonstrated any potential hazard of ultrasound on the early embryo in any of the species it has been used in so far in veterinary medicine.

As summarised in Table 3.1, two cows from group two conceived, though one of them had lost an earlier conceptus and then held to the next service. One cow from group two lost the conceptus and developed a luteal cyst. Three cows from the same group did not conceive, although one of the cows (cow 10) finally conceived after the end of the study. Cow 2 was not detected in heat at all during the observation period by the herdsman but the ultrasonic observation on day 30 indicated that the corpus luteum of the previous cycle had regressed and a new corpus luteum had been formed (Fig. 20e and 21e). This was further substantiated by the gradual decline

in plasma progesterone concentration around the time of luteal regression and then an increase corresponding to the formation of the new corpus luteum (Fig. 5a). There are possibilities that oestrus was short and went undetected or that the cow came in heat during the night when no one was observing the animals. This animal and a number of other repeat breeder cows had a high titre against both IBR and leptospirosis. Miller and Van Der Maaten (1985) found that IBR caused lesions in both the ovary and the uterus depending on the route of entry of the infection. In the ovary they found that it caused necrotising oophoritis that mainly affected the corpus luteum. As a consequence of the lesion they found that the plasma progesterone concentration was significantly depressed. In addition , they reported that chronic IBR infection caused severe sclerosis of the endometrium and this led to permanent sterility. Similarly, Ellis *et al.*(1976) observed that chronic leptospirosis infection also caused severe sclerosis of the endometrium and led to permanent sterility. In this study we observed that the corpus luteum of cow 2 had the smallest dimension compared to the rest of the animals. However, the plasma progesterone concentration did not vary much from the control group and in addition, the subsequent corpus luteum was much larger (Figure 5a). No attempt was made to assess the micro-morphology of the endometrium in this study thus it is difficult to comment on the observations made by Ellis *et al.*(1976) and by Miller and Van Der Maaten (1985). In addition, no literature is available on whether infection by IBR or leptospirosis causes any derangements in the neuroendocrine mechanisms controlling ovulation and corpus luteum formation. Peter *et al.*(1989) found that experimental *Escherichia coli* infection blunted the preovulatory LH surge and this could prevent ovulation. Further work is needed to clarify whether IBR or leptospirosis do in fact cause any neuroendocrine imbalance and if so to what

extent and whether the effect is temporary or permanent. Such information may provide a better explanation of how this cow remained open for approximately a year even though it was detected on heat several times (Table 3.1).

Cow 72 lost the conceptus before day 30 and returned to service (Fig. 6a and Fig. 27). It showed a gradual increase in plasma progesterone concentration compared to the cows in the control group which expressed a more rapid rise in plasma progesterone concentration (Fig. 1a). Even by day 16 the plasma progesterone concentration in this cow was still lower than that of the control group. A similar low progesterone concentration was seen up to day 10 in cow 14 but this cow did not conceive at all. Previous studies by Casida (1961), Bulman and Lamming (1978) and Maurer and Echternkamp (1982) associated failure to conceive with low plasma progesterone concentration but work by Kindhal *et al.*(1976), Linares *et al.*(1982) and Echternkamp and Maurer (1983) failed to find any association between low plasma progesterone concentration and pregnancy rates. Kodagali *et al.*(1979) and later work by Maurer and Echternkamp (1982) reported that low serum LH concentration was probably the cause of low luteal phase progesterone concentrations. Much recent work suggests that hormonal asynchrony produces an undesirable uterine environment for the embryo to survive (Pope,1988 & Roberts *et al.*,1990). Unfortunately, little information is available on how the composition of ions and other small molecules alter during the initial two weeks of pregnancy when the embryo remains unattached and essentially free living within the uterus. Linares *et al.*(1980) found a higher incidence of abnormal embryos in repeat breeder cows than in virgin heifers and there is a possibility that these abnormal embryos fail to provide adequate signals to extend the lifespan of the corpus luteum and maintain the progesterone production. During a second observation cycle in this study, cow 72 held to the service and the plasma progesterone concentration profile did not vary much from

that of the control group although it did perhaps show more variation from day 22 onwards than seen in the control group (Fig. 7a and Fig. 29). However, it should be emphasised that only a small number of animals were observed in this study and thus differences in plasma progesterone concentration in only two out of six repeat breeder cows makes it difficult to draw any conclusions as to the importance of progesterone in conception and its subsequent influence on repeat breeding.

A luteal cyst was observed in one repeat breeder cow (cow 1, Fig. 30f). It appeared to form from the follicle (Fig. 30e) between day 30 and 40 after insemination, to which the cow had apparently conceived. Although a proper embryo was not detected in this cow, the embryonic vesicle was identified (Fig. 32b, 32c and 32d). The subsequent enlargement of the vesicle and the maintenance of the corpus luteum on the ipsilateral ovary were convincing evidence that it had conceived. This is further substantiated by plasma progesterone concentration, which remained elevated up to the time when the embryo was lost and which then decreased rapidly to reach basal levels (Fig. 8a). The plasma progesterone concentration profile did not vary much from that of the control group up to the time the conceptus was lost. This animal had a titre against both IBR and leptospirosis and there is a possibility that the combined effect of the two infections had a profound negative effect on the dam and that it lost the conceptus. Hafez (1966) and Noakes (1988) reported that infections either cause direct embryonic death or they induce stress on the dam which too can result in embryo loss. However other cows with positive titres for IBR and leptospirosis did conceive and remained pregnant. The causes of cyst formation in cattle are still not clear but it is generally believed that an endocrine imbalance involving the hypothalamo-pituitary-ovarian axis leads to the condition (De Silvia and Reeves,1988). As mentioned earlier, IBR causes necrotic lesions in the ovary especially in the corpus luteum and this could cause an endocrine imbalance leading to

cyst formation. The ultrasonographic description of a luteal cyst as a non-echogenic area surrounded by a thick band of echogenic tissue is similar to that described by Edmondson *et al.*(1986) and Sprecher and Nebel (1988). Correct diagnosis of a cyst is pre-requisite for proper therapy and ultrasonography can be useful as a diagnostic tool with which to differentiate structures such as follicular and luteal cyst which feel similar and are therefore confusing on rectal palpation.

The key to successful breeding and reproductive performance is good management. Accurate heat detection is vital to the success of artificial insemination and achieving the 365 days calving interval. The efficiency of heat detection determines the conception rate and reduces repeat breeding. Heat detection on this particular farm was carried out six times a day by the herdsman. However, it always coincided with other chores such as feeding and milking. Williamson *et al.*(1972) reported that if heat detection was carried out by milkers, in addition to their chores, up to 12% of the cows presented for insemination may not actually be in heat. In addition, he pointed out that this figure increased to 36% if heat detection was done when animals were driven to and from the milking shed. Furthermore, Williamson *et al.*(1972) reported that 20% of cows displaying increased mounting activity are not actually in heat and are wrongly inseminated. O'Farrell (1975) recommended that checks must be conducted five times daily in an open yard with minimum disturbance of the animals in order to detect 80% of cows in heat. Although the average interval to first service of animals in group one was slightly longer than that recommended by Esslemont (1982), all four cows should be able to maintain a 365 day calving interval. In contrast, the cows in group two are unlikely to maintain a 365 day calving interval and this is a great economic loss and a setback for the farmer. From the number of services and days from calving to conception in group one and from the

number of times group two cows were observed in heat (Table 3.1), it is difficult to deduce whether poor oestrus detection is contributing to the repeat breeding problem on this farm. There certainly appeared to have been occasions when animals came in heat and were missed due to one reason or another. This is likely to have happened in cow 2 (Fig. 5a, 20 and 21) judging from the ultrasonic observations and plasma progesterone concentration profile.

Due to the small number of animals used and the limited number of reproductive criteria studied, it is difficult to draw any firm conclusions as to the precise cause of repeat breeding on this farm. However, the involvement of IBR and leptospirosis in the repeat breeding syndrome in this herd seems probable although further studies are needed to confirm this.

To conclude, transrectal ultrasonography in conjunction with measurement of plasma progesterone concentration provided a very useful means of monitoring reproductive function in both the normal and repeat breeder cows. Ultrasonography provided a non-invasive form of visual access to the ovaries and the uterus and allowed the detection and sequential monitoring of both follicles and corpora lutea. Measurement of plasma progesterone concentration had the added advantage of providing an indicator of ovarian function and, when used with ultrasonography, it provided a complete picture of reproductive status. This proved useful for early detection of pregnancy and for monitoring embryonic development and viability. Ultrasonography was safe and accurate and further use of it will hopefully provide more pertinent information on the critical period of embryonic death in cattle. Though currently the cost of the equipment precludes the complete replacement of bovine rectal palpation by ultrasound, ultrasonography can augment rectal palpation and endocrinology in individual

animals that present diagnostic problems. Further studies are needed to confirm, clarify and extend the findings reported and to justify the commercial use of ultrasonography in monitoring herd fertility.

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